

Female Voice-Related Sexual Attractiveness to Males: Does it Vary With Different Degrees of Conception Likelihood?

*[†]Filipa M.B. Lã, *[‡]Nuria Polo, ^{§,||}Svante Granqvist, ¶[¶]Tânia Cova, and ¶[¶]Alberto C. Pais, *[‡]Madrid, Spain, †[¶]Coimbra, Portugal, and §^{||}Huddinge, Sweden

SUMMARY: Previous investigations have found that female voice-related attractiveness to males increases when both conception likelihood (CL) and voice fundamental frequency (f_0) are elevated. To test this hypothesis, we conducted a perceptual experiment where 78 heterosexual males rated sexual attractiveness of 9 female voice samples, recorded at menstrual, follicular and luteal phases of the menstrual cycle under two double-blinded randomly allocated conditions: a natural menstrual cycle (placebo condition) and when using an oral contraceptive pill (OCP condition). The voice samples yielded a total of 54 stimuli that were visually sorted and rated using Visor software. Concentrations of estrogens, progesterone and testosterone were analyzed, and measurements of speaking fundamental frequency (sf_0) and its standard deviation (sf_0SD), f_0 derivative (df_0) and f_0 slope were made. A multilevel ordinal logistic regression model nested in listeners and in females, and adjusted by phase and condition, was carried out to assess the association between ratings and: (1) phases and conditions; (2) sex steroid hormonal concentrations; and (3) voice parameters. A high probability of obtaining high ratings of voice sexual attractiveness was found for: (1) menstrual phase of placebo use and follicular phase of OCP use; (2) for low estradiol to progesterone ratio and testosterone concentrations; and (3) for low df_0 . The latter showed a moderate statistical association with ratings of high attractiveness, as compared with the small association found for the remaining variables. It seems that the voice is a weak cue for female CL. Female sexual attraction to males may be a consequence of what females do in order to regulate their extended sexuality across the menstrual cycle rather than of estrus cues, the use of paralinguistic speech patterns being an example.

Key Words: Female voices—Sexual attractiveness—Menstrual cycle—Oral contraceptive pill—Sex steroid hormones—Fundamental frequency.

INTRODUCTION

The human voice conveys important physical, psychological and social information about the speaker,¹ regardless of linguistic and emotional contents.² From an aesthetical point of view, the perception of voice quality has been linked to “beauty” and “attractiveness,” two related concepts that however can be dissociated.³ In the present study, voice-related attractiveness is the focus of attention, investigated from a perspective of mate selection choices.³ In previous research, certain acoustical characteristics of the voice have been claimed to identify when females are most fertile

during the menstrual cycle.⁴ The aim of this study is to explore perceptions of female voice-related sexual attractiveness to males across different phases of the menstrual cycle and under different degrees of conception likelihood (CL).

Sex steroid hormones, estrogens (E), progesterone (P) and testosterone (T), have a key-role in human reproductive physiology and behavior.⁵ These same hormones are also responsible for sex-dependent voice characteristics⁶ and have been associated with voice-related sexual preferences.⁷ Thus, relationships between variations in concentrations of sex steroid hormones across the menstrual cycle and female voice-related sexual attractiveness to males can be expected. For instance, female voices have been rated as more sexually attractive when CL is higher.^{8,9} When using an oral contraceptive pill (OCP), that is, when CL is significantly reduced or even absent, female voice-related sexual attractiveness to males was found to be low.⁹ Based on these findings and those of Puts et al. (2013), the current study hypothesizes that female voice-related sexual attractiveness to males is low when CL and concentrations of estradiol (E_2), the most common E, are both low. To test this hypothesis, we assess female voice-related sexual attractiveness to males at different phases of the menstrual cycle and at different CL conditions, using voice samples of women when using an OCP and a matched placebo, samples taken from a previous double-blind randomized placebo controlled trial (RCT).¹⁰ First, we explore how sex steroid hormones and their cyclical variations through phases of the menstrual cycle may affect histological characteristics of the vocal

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From the *Faculty of Education, National University of Distance Learning, Madrid, Spain; †Centre of Social Studies, University of Coimbra, Coimbra, Portugal; ‡Faculty of Philology, National University of Distance Learning, Madrid, Spain; §KTH Royal Institute of Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health (CBH), Department of Biomedical engineering and Health systems, Karolinska University Hospital, KTH Royal Institute of Technology, Huddinge, Stockholm, Sweden; ||Karolinska Institute, Department of Clinical Science, Intervention and Technology (CLINTEC), Division of Speech and Language Pathology, Huddinge, Stockholm, Sweden; and the ¶Coimbra Chemistry Center, University of Coimbra, Coimbra, Portugal.

Address correspondence & reprint requests to: Filipa M.B. Lã, Faculty of Education, National University of Distance Learning, Calle Juan del Rosal 14, 28040 Madrid, Spain E-mail: filipa.la@edu.uned.es

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folds and acoustical properties of the voice. In addition, we debate previous findings on female sex hormones and potential impacts on acoustic metrics, namely fundamental frequency (f_0) and perceptions of female voice-related sexual attractiveness to males.

Voice characteristics throughout the menstrual cycle

Receptors for sex steroid hormones have been found in several sub-units of the vocal folds mucosa.¹¹ For both female and male vocal folds, it seems that receptors for androgens (A) and E_2 are the most abundant.¹¹ However, the percentage of cells that constitute the mucosa of the vocal folds may vary, depending on sex. For females, this percentage varies across the menstrual cycle, mimicking the changes observed in the percentage of cells that constitute the mucosa of the cervix.¹² Conversely, for males, this percentage remains constant throughout the whole month.^{12,13} Moreover, as female concentrations of sex steroid hormones vary cyclically throughout the month, the hormone-receptor interactions may differ in females. One may then expect an enhanced effect of sex steroid hormones on female laryngeal tissues as compared to males.¹⁴

Figure 1 represents sex steroid hormonal variations across a 30-day menstrual cycle. This cycle can be divided into three phases: menstrual, follicular and luteal.¹⁵ At menstrual phase, also referred as the ischemic phase of the menstrual cycle, concentrations of E_2 , P and T are low. The follicular phase is regarded as the fertile phase of the cycle: the preovulatory surge in LH and the subsequent rise in E_2 , provide good estimates of conception capacity.¹⁶ Often, the follicular phase is divided into early follicular (preovulatory) and late follicular (postovulatory).^{17,16} In addition, a small increase in T concentrations can also be detected during

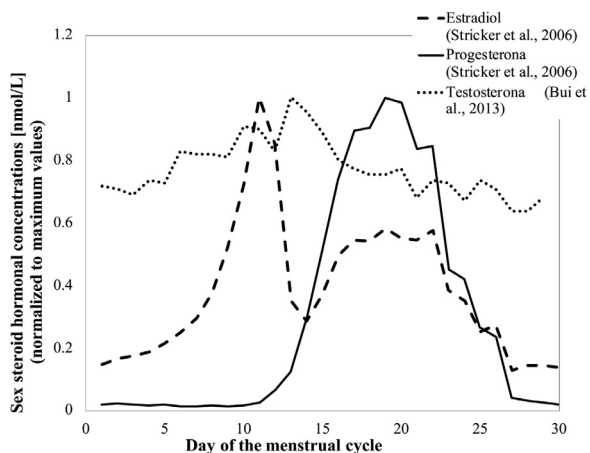


FIGURE 1. Sex steroid hormonal variations across a natural 30-days menstrual cycle, divided into three phases: menstrual, follicular, and luteal phases. Concentrations of testosterone (dotted line), estradiol (dashed line) and progesterone (solid line) were normalized to maximum values and expressed in the same unit (nmol/L) for purposes of comparisons (data retrieved from Buit et al, 2013 for testosterone concentrations¹⁸ and from Stricker et al, 2006 for estradiol and progesterone concentrations¹⁹).

this phase. The luteal phase is characterized by a peak in P concentrations, with a small rise in E_2 and a fall in concentrations of T. At the end of this phase, concentrations of all sex steroid hormones decrease, leading to menstruation and, with it, the beginning of a new menstrual cycle.

The constant variations in concentrations of sex steroid hormones verified during a natural menstrual cycle have been associated with several vocal symptoms, including changes in mean f_0 and related perceived pitch. However, the exact mechanisms by which these hormonal variations impact on f_0 and pitch are still debated. For example, results of a RCT investigating f_0 variations across the menstrual cycle and when using an OCP suggest that f_0 values are lower at the menstrual phase as compared to other phases of the menstrual cycle.²⁰ However, no differences in f_0 between phases of the cycle were found in other previous investigations.^{21,22} When assessing effects of specific concentrations of sex steroid hormones on mean f_0 , the results are also controversial. For instance, a high correlation between f_0 and concentrations of E_2 was found in some studies,²³ whereas others could not find such relationship.²⁴

Fundamental frequency and female voice-related sexual attractiveness to males

Vocal cues are determinant when visual stimuli are unclear or absent.²⁵ Previous investigations have claimed that f_0 , for example, can favor sexual preferences in humans.^{26–29} The most accepted theory has been that men prefer women with higher f_0 . The rationale is that this acoustical feature is associated with more feminine and young bodies,³⁰ such as smaller body mass and height.³¹ However, controversy exists concerning which female f_0 values could be considered as more sexual attractive to males or even if f_0 alone is a strong predictor of sexual attraction. First, one study has found no correlation between f_0 and attractiveness.³² Second, a study on speed dating revealed that males found female voices more attractive when their speaking pitch was reduced by about 1.5 semitones (ST), that is, when f_0 was reduced by about 15.8 Hz.³³ Third, another study claimed that female voices were perceived as more attractive when f_0 fell within the range of 184 Hz to 262 Hz (F#3 to C4).³⁴

The complex nature of female voice-related sexual attractiveness to males increases even more when mean f_0 is used as a predictor of female ovulation, of CL, or of fertility status. Although ovulation, CL and fertility are related, they should not be regarded as synonyms. Ovulation is a single-day event during the follicular phase of the cycle (therefore, also considered as the fertile phase); it can be detected accurately only by ultrasonography. Fertility and CL, on the other hand, can last several days during the follicular phase of the cycle, depending on sperm and oocyte lifespans, and both can be detected by day of the cycle (providing that it is a regular one) and by a surge in concentrations of LH and estradiol.¹⁶ Bryant and Haselton (2009) found that when female fertility is high, pitch is also perceived as being high.⁴ However, such association could not be replicated in other

studies. For instance, despite perceptions of female voice-related sexual attractiveness to males were higher at the fertile phase of the cycle when compared to other phases, such perceptions were found to be associated with low f_o values.³² Moreover, differences in f_o could not be found when comparing fertile with non-fertile phases of the menstrual cycle.⁷

Relationships between ratings of female voice-related sexual attractiveness to males, specific concentrations of sex steroid hormones across the menstrual cycle and f_o were also investigated. Results showed that the higher the E_2/P , the higher the female voice-related sexual attractiveness. However, both ratings and f_o measures were observed when comparing different female voices. The current investigation presents a dataset drawn from a RCT where both voices and blood samples were collected from the same female, at three phases of the menstrual cycle and at two conditions, i.e., placebo and OCP.¹⁰

MATERIAL AND METHODS

Female voices

Participants for voice recordings were nine white European females (mean age = 23.1 yrs.; SD = 2.183; age range = 21 - 27 yrs.), all healthy volunteers reporting normal and regular menstrual cycles. They were students of singing at different higher educational institutions in the United Kingdom. Ethics approval was obtained from the South Sheffield Ethics Committee (SSREC/02/028) as described elsewhere.¹⁰ All participants were assessed for fulfilment of inclusion criteria: to be non-smoker, have no history of vocal pathology, asthma, reflux and pulmonary disease, and be suitability for OCP use.

Female hormonal and voice samples

Participants were given identical numbered packs of placebo and OCP for a total of 6 months. Both placebo and OCP were taken for 21 consecutive days, with a 7-day interval between packs and during 3 consecutive months. The study started on the first day of the menstrual cycle, when the first pill of the six identical packs (i.e., one per month) was taken.

Five subjects were randomized to take the placebo during the first three months of the study, and four to take OCP. This randomization was done blinded to both researchers and participants to: (1) reduce the risk of outcomes being influenced by expectations of both researcher and participant about the experimental drug³⁵; (2) provide similar experimental conditions for all participants, and thus reduce biases due to external factors and individual differences³⁶; and (3) allow within-subject comparisons, an important consideration when carrying out perceptual evaluations of voices and when using small sample sizes.³⁷ A 55% correct placebo and/or OCP guess was found at the end of the experiment. This result is substantiated by previous RCTs percentages on participant's blindness integrity.³⁸

The OCP preparation was *Yasmin* (Schering Health Care Ltd.), a combined monophasic OCP containing 30 μ g of

ethinylloestradiol and 3 mg of drospirenone. The choice of this OCP was related to the fact that it contained low doses of synthetic hormones. In addition, this OCP has been reported to be well-tolerated due to its androgenic and corticosteroid properties³⁹. A monophasic combined OCP was chosen instead of a triphasic one because the latter does not provide constant concentrations of hormones across the menstrual cycle.¹⁵

Both blood samples and voice recordings were collected at menstrual, follicular and luteal phases of the third menstrual cycle for placebo and for OCP use. Data collection were planned according to participants' reports on previous menstrual cycles' durations. This was possible because all participants had regular menstrual cycles of similar durations. Days 1 and 2 of menstruation were regarded as the first day of the menstrual cycle and representative of the menstrual phase, whereas days 8 to 10 and 22 to 27 (depending on individual cycle length) were representative of the follicular and luteal phases, respectively.⁴⁰

Blood samples were taken to ensure correct use of both placebo and OCP and to measure concentrations of E_2 , P, T and E_2/P , measured at the Department of Clinical Chemistry at the Royal Hallamshire Hospital in Sheffield, UK. Further descriptions on procedures for serum hormonal analysis can be found elsewhere.¹⁰

Simultaneous recordings of audio and electrolaryngographic (ELG) signals were made while participants read the *Rainbow Passage*.⁴¹ This is a standard phonetically balanced text commonly used to study acoustic properties of the voice in speech, such as f_o and related parameters. From these voice recordings, the phrase "People look, but no one ever finds it" was extracted. This particular phrase was chosen because it is located at approximately one third of the whole text. Thus, the reader would have sufficient time to get accustomed to the task, without lacking attention nor being tired.⁴² A phrase was chosen instead of a sustained vowel because samples containing words were found to be more sensitive to ratings of attractiveness than isolated vowels.⁴³ In addition, connected speech provides more complete acoustic information than sustained vowels.⁴⁴ Finally, studying a phrase rather than a vowel allowed extraction of several acoustical parameters crucial to voice and speech perception, such as mean f_o taken from speech, hence, speaking fundamental frequency (sf_o), its variation and contour.⁴⁵

All recordings were made using: a MBNM550E-L omnidirectional microphone (Canford Audio, Washington, Tyne and Wear, United Kingdom) placed off-axis 30 cm from the participant's lips; an Alice mic-amp-pak1 preamplifier (Alice Soundtech Ltd., Surrey, United Kingdom); a two channel TCD-D7 stereo digital audio tape recorder (DAT) (Sony, Tokyo, Japan); a portable electrolaryngograph (Laryngograph Ltd., London, United Kingdom), which uses two neck electrodes held in place externally to the thyroid notch by an elastic neck band to measure vocal folds contact; and a portable oscilloscope displaying the electrolaryngograph output signal (ELG) to ensure appropriate electrode placement.

Both audio and ELG signals were used in the current study. The first provided the stimuli to be rated in the perceptual experiment; the second allowed the extraction of f_0 and all related parameters. The extraction of these parameters from ELG signals allows f_0 measurements only when the vocal folds are in contact. Such procedure provides data voided of possible acoustic artifacts resulting from environmental noise and/ or room acoustics observed in audio signals when correct choice of microphone and microphone placement are not taken into account.⁴⁶ Audio and ELG signals were separated into two channels. Audio signals were normalized for sound level and saved separately as .wav files for later use in the perceptual experiment.

Speaking fundamental frequency and related measures

The following acoustic parameters were extracted from ELG signals: (1) sf_0 ; (2) f_0 variation measured as sf_0 standard variation (sf_0SD); (3) the speed of f_0 change or, in other words, f_0 derivative (df_0); (4) and the declination of f_0 contour (f_0 slope). These measures were chosen because of their relationship to perceived pitch and prosodic variation, two key elements when perceptually evaluating a voice.⁴⁷

Extraction of f_0 and related measures was made by means of the *Corr* correlogram tool available in the software *Sopran* (freeware at www.tolvan.com). This tool displays a three-dimensional graph showing the periodic characteristics of the voice. The f_0 is traced manually and the software extracts f_0 values within the traces corresponding to the highest correlation. Fundamental frequency values are therefore free from an automatic selection mechanism of f_0 and perturbation measures.⁴⁸ The output is the f_0 curve in a .SMP format signal file (see Figure 2), from which f_0 values can be exported to a spreadsheet file.

Speaking fundamental frequency was computed in the excel file together with sf_0SD . The df_0 was calculated as the absolute difference between two adjacent frequencies in a time window of 10 milliseconds. The f_0 slope was extracted from f_0 contour, following Lieberman's *et al.* (1985) method.⁴⁹

Male raters

A total of 328 males volunteered to take part in the perceptual experiment. Participants were recruited through authors' pre-existing contacts. Inclusion criteria were: (1) heterosexual men; (2) older than 18 years old; (3) no hearing impaired; (4) non-native English speakers. Heterosexual males were considered eligible as previous studies identified predictions of voice-related sexual preferences based on perceptual analysis of the voice as more robust when made by participants of a different sex.^{3,25} Only non-native English speakers were included as raters because a foreign accent may influence voice-related sexual preferences.⁵⁰

Because this was a multiple research center investigation, ethics approval to conduct the perceptual experiment was obtained from two ethical committees: (1) Ethical Committee at the Faculty of Psychology and Education Sciences, University of Coimbra, Portugal; and (2) Ethical Committee of the National Distance Learning University in Madrid, Spain.

Perceptual experiment

Raters who met the inclusion criteria and who gave back a signed consent form ($n = 111$) were given access to a downloadable version of the software *Visor* (Granqvist 2003; freeware at www.tolvan.com). To guarantee listener's confidentiality and anonymity of their responses, an automatic ID code was generated. Since *Visor* is not compatible with tablets or mobile phones, raters were asked to use a personal computer.

Visor builds listening tests containing stimuli in free order; evaluations consist of ranking and visually sorting the stimuli presented as icons on a computer screen. This specific computer program was chosen because it increases inter- and intra- subject correlations: each stimulus acts as an external reference of similar perceptual value to the one evaluated without the need for anchor stimuli.⁵¹ Because raters can directly listen to the difference between two closely rated stimuli and adjust their responses, this method also improves the rank ordering ability of the evaluators. Possible sources of errors when dealing with large datasets

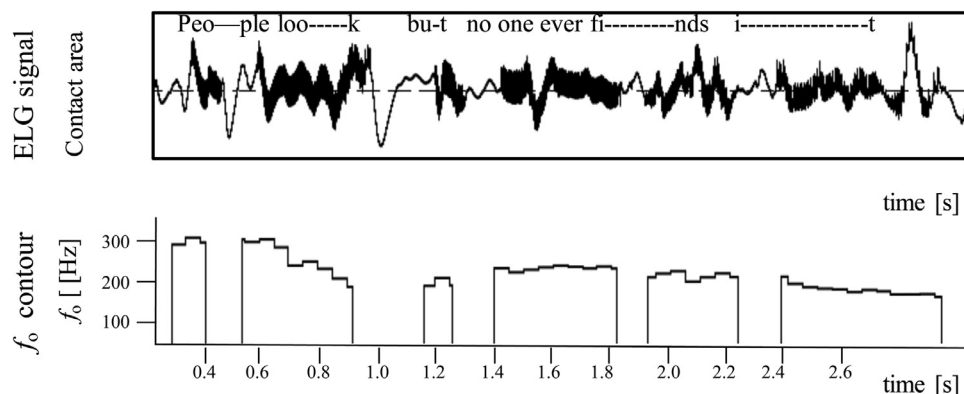


FIGURE 2. Fundamental frequency (f_0) contour (lower panel) extracted from the ELG signal (upper panel) which displays vocal fold contact area during speech.

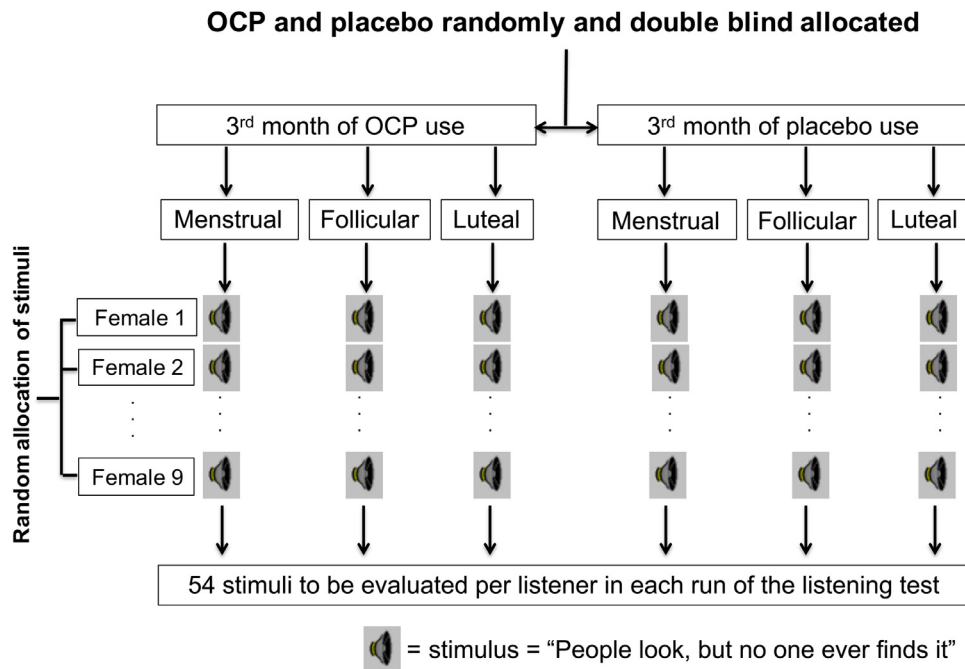


FIGURE 3. Visual representation of the perceptual experiment design used in each of the two runs of the perceptual experiment.

are also reduced using Visor, as manually entering of data is avoided.⁵¹

As there were nine female voices to be rated, the six stimuli belonging to each voice were displayed in nine consecutive Visor windows: the same phrase recorded by the same female at three phases of the menstrual cycle for both placebo and OCP conditions. Once the six stimuli of one window were played back as many times as necessary, they were visually sorted and rated and results were saved. Then a new window would pop-up containing 6 other stimuli to be rated, these now belonging to another of the nine female voices. Both Visor stimuli and the results of the listening evaluations were saved on a ftp server located at Centre for Social Sciences, at the University of Coimbra (<http://ftp.ces.uc.pt>). These were made accessible only to the researchers for later analysis.

Listeners were asked to run this perceptual experiment twice (hence runs A and B). These runs differed in the order of presentation of female voices per Visor window and in the order of the stimuli presented in each window. Randomly allocation of voices and stimuli in both runs were automatically made by Visor. Such procedure was done to assess whether ratings would be capable of producing reliable scores in terms of stability and reproducibility.⁵²

Raters were asked to complete both runs A and B in a quiet room, using headsets, and in two consecutive days. Each listener sorted and rated 54 stimuli in each of the two runs: six audio excerpts to rank and order at a time, corresponding to three phases of the menstrual cycle and two conditions, placebo and OCP, repeated nine times (as there were nine different female voices). Thus, each listener would have to complete 108 ratings (6 stimuli per voice x 9 female voices x 2 runs). Given that similar result patterns are

obtained from both online and presential listening tests,⁵³ the ratings were done either on a Lab or at home. A summary of the perceptual experimental design for one run is displayed in Figure 3.

Analysis of male ratings

The results of sorting and rating the stimuli in Visor were uploaded in the ftp server as a continuous scale (from 0 to 1000 points). However, as each evaluator was requested to sort and order 6 stimuli per window, the absolute scores had to be converted into ordinal ranks from 1 to 6, 1 being not at all sexually attractive and 6 being very much sexually attractive (see Figure 4).

Concordance of rank responses between runs A and B of the listening test was verified by Cohen's kappa. This coefficient was chosen to test intra-rater reliability between both runs. A descriptive analysis of all collected data (hormonal and voice parameters) was performed. The frequency of the ratings per rank was determined, for each phase of the menstrual cycle, and for both placebo and OCP conditions. The results were summarized using measures of central tendency according to the distribution of the variables.

To test the hypothesis that ratings of female voice-related sexual attractiveness to males were associated with phase of the menstrual cycle and condition, a multilevel ordinal logistic regression model was carried out, nested in listeners and females. Odds ratios (OR) and 95% confidence intervals were used to determine the effect size of the association between stimuli rank order and (1) phase of the menstrual cycle (menstrual, follicular and luteal), and (2) condition (placebo or OCP). An OR of approximately 1.6 was considered as a small effect size; between 3.5 and 6.7 the effect size

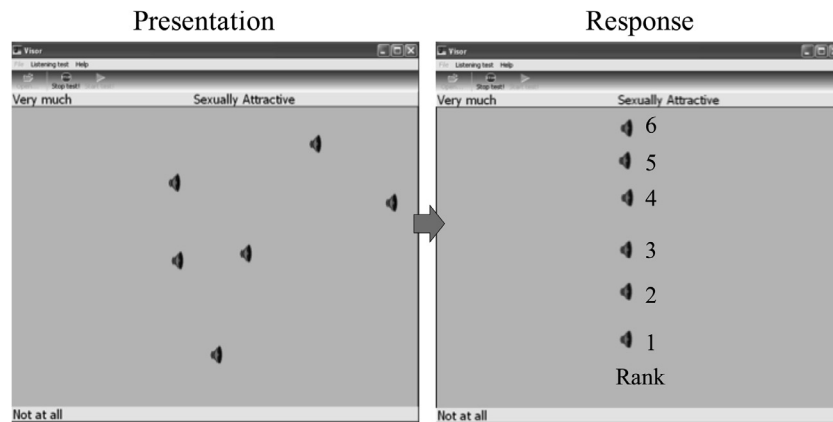


FIGURE 4. Example of the stimuli belonging to one female voice displayed in a *Visor* window. Each of the 6 loudspeaker icons correspond to a voice excerpt containing the phrase “People look but no one ever finds it” spoken by the same female at three phases of the cycle and two conditions (ie, placebo and OCP). Stimuli were ordered and ranked according to the listener’s perception of degree of voice-related sexual attractiveness, ranging from not at all sexually attractive (bottom of the computer screen, rank 1) to very much sexually attractive (top of the computer screen, rank 6).

was considered moderate; and when bigger than 6, it was considered large.⁵⁴ The selected reference category was the menstrual phase of placebo use, since this phase corresponds to the non-fertile and low CL phase of the menstrual cycle, regardless of OCP use. A forward selection model was performed with the remaining variables, i.e., sex steroid hormonal concentrations collected for each phase and condition, and f_0 and related parameters. This model was applied to further explore whether distribution of evaluations for each rank could be affected by sex steroid concentrations and/ or by f_0 and related measures. A change of scale was made for those parameters requiring clear interpretation of effect size. The statistical analysis included both listeners and females as a random effect. All analyses were performed with the Stata v.14.2 statistical software (Stata Corp. 2015. Stata Statistical Software: Release 14). All tests were carried out at a significance level of $\alpha = 0.05$.

RESULTS

Female voice characteristics and sex steroid hormonal concentrations

To confirm that voice and hormonal data collected at different phases of the menstrual cycle were representative of different degrees of CL, the days of the menstrual cycle in which data were collected were computed as a percent of CL. This was made by applying reference values taken from a previous investigation where CL was estimated as a single unprotected intercourse during a regular menstrual cycle; the highest CL for a natural regular menstrual cycle was 10.1%⁵⁵. The results confirm that data collected in the current investigation were collected at different degrees of CL, ranging from low (0.1 %), to high (8.8 %) (see Figure 5).

As data are not normally distributed, the median (Mdn) and first and third interquartile ranges (IQR) for both sex steroid hormonal concentrations and voice parameters are displayed in Table 1, presented separately for each phase of the

menstrual cycle for the placebo condition. Likewise, Table 2 displays Mdn and IQR for both sex steroid hormonal concentrations and voice parameters, presented separately for each phase of the menstrual cycle, for the OCP condition.

Ratings of female voice-related sexual attractiveness to males

Of the 111 men participating in the perceptual experiment, only 78 (mean = 33.4 ± 12.2 ; age range = 19 to 61 years old) successfully completed the whole experiment (ie, completed both runs A and B in *Visor*). A total of 8424 evaluations were thus obtained: 78 listeners ranking 54 stimuli (9 female voices for 3 phases and 2 conditions), repeated twice. From these evaluations, 282 stimuli in run A and 306 in run B were not valid; they corresponded to about 6.7% and 7.3% of missing values for runs A and B, respectively, and thus were excluded from further analysis. The total number of stimuli included in the statistical model was 7836.

Concordance of ratings between runs A and B was statistically significant, although small: 25.6 % of agreement (Cohen’s kappa = 0.1071, $P < 0.0001$). For the purposes of this investigation, only the results of run B are presented, the rationale being three-fold. First, the sensitivity analysis performed on run A shows similar results and trends to those obtained from run B. This confirms that the choice of runs does not influence the results. Second, the distribution of ratings is similar for both runs A and B. Third, one may consider run A as a training session during which the rater becomes acquainted with *Visor* and with the stimuli. When raters are trained for a perceptual test, their ratings are more robust⁴². One might also argue that run A would have yielded more reliable ratings as compared to run B because run A is free from a possible learning effect on ratings⁴⁷. However, such effect is circumvented in run B with the randomization of the stimuli, for both within and between voices’ comparisons, and by one-day separation between runs.

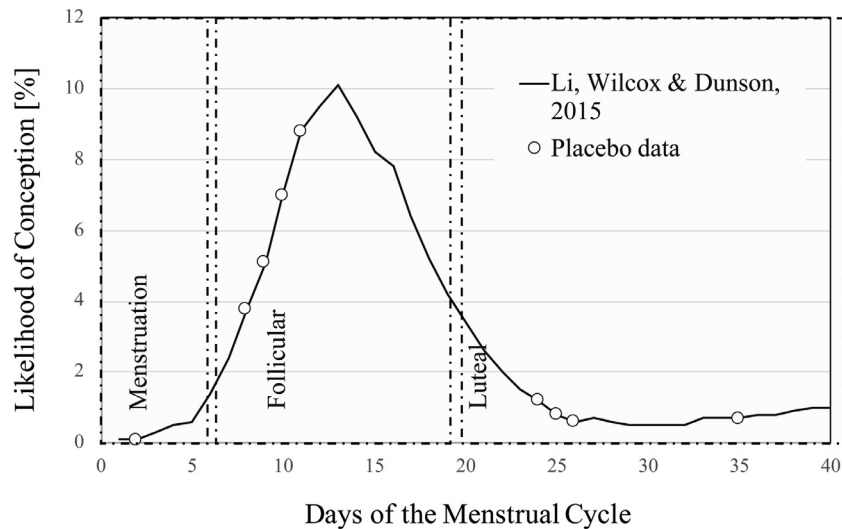


FIGURE 5. Conception likelihood (CL) as a function of days of a regular menstrual cycle. The solid line corresponds to data retrieved from a previous investigation⁵⁵ used to compute CL for the present investigation. Open circles represent data collection points for the placebo condition of the present investigation, for both voice and hormonal data. The menstrual cycle was divided into three phases, menstrual, follicular and luteal, following previously established cycle divisions¹⁵.

Figure 6 shows the distribution of rankings, for all phases and conditions. For the menstrual phase of placebo use and for the follicular phase of OCP use, the frequency of the ratings is clearly higher for rank 6. By contrast, the luteal phase of OCP presents relatively fewer ratings at rank 6. The remaining phases show a uniform distribution for both conditions.

Results of the multilevel ordinal logistic regression analysis concerning the association between ratings, phase, condition, and the combination of both, revealed that the probability for obtaining a high rank is different in all phases and conditions, except for menstrual and follicular phases of placebo and OCP use, respectively (see Table 3). In both cases, the probability of obtaining a high rank is similar and the effect size small and negative.

With respect to the association between ratings and sex steroid hormonal concentrations, Figure 7 provides a summary of the results. It can be observed that the lower the E_2/P and the T concentrations the higher the probability for female voice-related sexual attractiveness to males.

The results of the statistical model showed that only E_2/P and T present a statistically significant association (see Table 4), but the effect size is small and negative. The higher the E_2/P or T concentrations, the lower the probability of obtaining a high rank.

As to what concerns the association between ratings and voice parameters, only df_o presents a significant statistical association (see Table 5). Here, the effect size is medium-large and negative, which means that the higher the df_o , the lower the probability of obtaining a high rank.

TABLE 1. Median (Mdn) and Interquartile Range (IQR) for Both Sex Steroid Hormonal Concentrations and Female Voice Parameters for Placebo Use

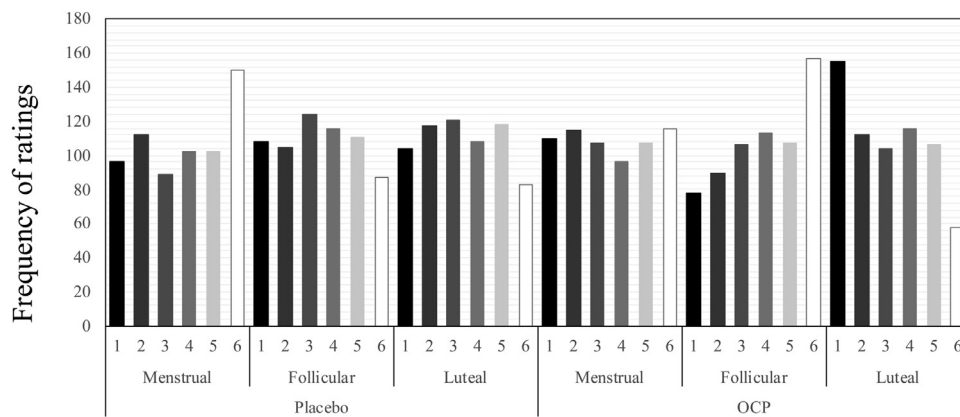
	Placebo		
	M Mdn (IQR)	F Mdn (IQR)	L Mdn (IQR)
E_2 [pmol/L]	130 (109: 168)	196 (113: 463)	437 (284: 579)
P [nmol/l]	3 (2.3: 4.3)	2.5 (2: 3)	30.7 (20.5: 40.6)
E_2/P	40.4 (33.03: 78.8)	64.6 (39.2: 257.2)	14.4 (10.3: 17.9)
T [nmol/L]	1.5 (1.2: 1.7)	1.8 (1.8: 2.2)	1.7 (1: 1.9)
sf_o [Hz]	239.7 (220.9: 245.8)	232.4 (225.5: 267.0)	240.4 (210.3: 274.7)
sf_oSD [Hz]	32.9 (28.4: 39.6)	41.9 (30.3: 46.9)	40.6 (32.6: 46.7)
df_o [Hz/s]	0.1 (0.1: 0.2)	0.1 (0.1: 0.2)	0.2 (0.1: 0.2)
f_o slope	-52.0 (-59.3: -40.2)	-72.2 (-91.4: -56.7)	-55.6 (-101.1: -32.5)

M, menstruation; F, follicular; L, luteal; E_2 , estradiol; P, progesterone; E_2/P , estradiol-progesterone ratio; T, testosterone; sf_o , speaking fundamental frequency; sf_oSD , fundamental frequency standard deviation; df_o , fundamental frequency derivative; f_o slope, fundamental frequency slope.

TABLE 2. Median (Mdn) and Interquartile Range (IQR) for Both Sex Steroid Hormonal Concentrations and Female Voice Parameters for OCP Use.

	OCP		
	M Mdn (IQR)	F Mdn (IQR)	L Mdn (IQR)
E [pmol/L]	82 (61: 105)	73 (64: 111)	56.9 (57: 69)
P [nmol/l]	1.8 (1.5: 3)	3.3 (2.4: 5.9)	2.1 (1.9: 3)
E ₂ /P	367.0 (33.9: 63.3)	22.1 (15.3: 37.5)	28.7 (20.3: 31.6)
T [nmol/L]	1.2 (0.7: 1.3)	1.1 (1: 1.6)	0.9 (0.7: 1.5)
sf ₀ [Hz]	227.4 (221.9: 256.4)	239.5 (212: 259.3)	235.2 (211.2: 261.7)
sf ₀ SD [Hz]	47.7 (35.5: 51.8)	33.02 (26.32: 37.9)	32.8 (27.9: 43.4)
df ₀ [Hz/s]	0.2 (0.1: 0.2)	0.1 (0.1: 0.1)	0.2 (0.1: 0.8)
f ₀ slope	-56.9 (-80.1: -48.8)	-62.6 (-70.0: -41.1)	-40.2 (-53.3: -14.7)

M, menstruation; F, follicular; L, luteal; E₂, estradiol; P, progesterone; E₂/P, estradiol-progesterone ratio; T, testosterone; sf₀, speaking fundamental frequency; sf₀SD, fundamental frequency standard deviation; df₀, fundamental frequency derivative; f₀ slope, fundamental frequency slope.



Not at all sexually attractive ■ 1 ■ 2 ■ 3 ■ 4 ■ 5 ■ 6 Very much sexually attractive

FIGURE 6. Distribution of voice-related sexual attractiveness per phase - menstrual, follicular and luteal - and condition – placebo and OCP. The bars represent the frequency of each rank according to participants’ preferences of voice-related sexual attractiveness (1 = not at all sexually attractive to 6 = very much sexually attractive).

TABLE 3. Effect Size for the Association Between Ranks and Phase, Condition, and the Combination of Both, Nested by Listener and Female

	Effect size		
	OR	95% CI	P-value
Condition-Phase (ref: Placebo-Menstruation)			
Placebo-Follicular	0.75	0.62, 0.91	0.004*
Placebo-Luteal	0.74	0.61, 0.90	0.002*
OCP-Menstruation	0.81	0.66, 0.98	0.031*
OCP-Follicular	1.17	0.96, 1.42	0.117
OCP-Luteal	0.55	0.45, 0.67	0.0001*

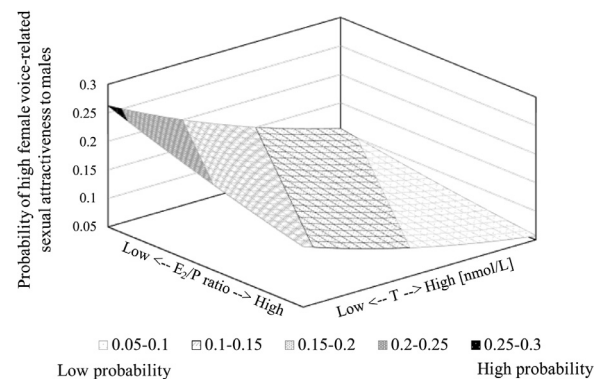


FIGURE 7. Probability of high female voice-related sexual attractiveness to males (the darker the shade of green, the higher the probability) as a function of low E₂/P and low T concentrations. T = testosterone; E₂/P = E₂ to P ratio.

TABLE 4.
Effect Size for the Association Between Rankings and Sex Steroid Hormonal Concentrations, Adjusted by Phase, Condition and the Combination of Both, and Nested by Listener and Female

	Effect size		
	OR	95% CI	P-value
E_2^+	1.04	0.98, 1.11	0.168
P^+	0.69	0.24, 1.97	0.484
E_2/P^+	0.88	0.78, 0.98	0.026*
T	0.79	0.69, 0.90	0.001*

N.B.: +divided by 100

E_2 , estradiol; P, progesterone; T, testosterone; E_2/P , ratio between E_2 and P.

TABLE 5.
Effect Size for the Association Between Rankings and Voice Parameters, Adjusted by Phase, Condition and the Combination of Both, and Nested by Listener and Female

	Effect size		
	OR	95% CI	P-value
sf_o^+	0.93	0.63, 1.38	0.709
sf_oSD^+	1.72	0.64, 4.62	0.284
df_o	0.17	0.04, 0.70	0.014*
f_o slope ⁺	0.79	0.60, 1.04	0.088

N.B.: +divided by 100

sf_o , speaking fundamental frequency; sf_oSD , fundamental frequency standard deviation; df_o , fundamental frequency derivative; f_o slope, fundamental frequency slope.

DISCUSSION

This study aimed at exploring relationships between sex steroid hormones and voice-related preferences as to what concerns female voice-related sexual attractiveness to males across different phases of the menstrual cycle and under different degrees of CL. Given the results of previous research, the null hypothesis was that female voice-related sexual attractiveness to males is low when CL and concentrations of E_2 are both low. A perceptual experiment was carried out: 78 heterosexual males rated female voice-related sexual attractiveness in voice samples taken from 9 women. These samples consisted of a phrase taken from *The Rainbow Passage*, recorded at three phases of the menstrual cycle - menstrual, follicular and luteal - under two conditions: natural and hormonal artificially dampened menstrual cycles. Such study design was carried out to ensure comparisons of ratings within the same female voice at different degrees of CL. Both concentrations of sex steroid hormones and acoustic parameters were measured for all phases and conditions.

The results of the blood samples confirmed that concentrations of E_2 , P and T were representative of menstrual, follicular and luteal phases and of correct OCP use.⁵⁶ As expected, IQRs were larger during placebo as compared to

OCP use; the latter dampens hormonal variations across the menstrual cycle, as expected for a monophasic combined contraceptive pill.¹⁵ Thus, data were representative of different phases and CL conditions for the stimuli rated. To minimize possible effects of great IQRs variability in hormonal concentrations between placebo and OCP conditions on ratings of voice attractiveness, a multivariate logistic regression was made, as it controls for factors that could change or modify the results. Because these factors are controlled in multivariate models, the final results show the “real” association without “noise”.

Ovulation was not included in our dataset. Ovulation occurs at one specific day of the cycle; thus, to detect ovulation, ultrasonography is needed.¹⁶ An alternative to ovulation is the identification of the fertility window within the menstrual cycle, when CL is also high. According to Li et al. (2015), for a regular menstrual cycle, CL varies between 0.001%, at menstrual phase of the cycle, and 10.1%, around ovulation. Following this same CL estimation, the dataset for the follicular phase of placebo condition were collected at a CL of 8.8%. Therefore, one may argue that, although ovulation was not included, a high CL was still ensured. Given the life span of sperm (3 to 5 days before ovulation), and the oocyte lifespan (1 to 2 days after ovulation),¹⁶ one must consider that the days in which our data were collected for the follicular phase of placebo use correspond to a high CL window.

The present results suggest that the probability of high female voice-related sexual attractiveness to males existed at menstrual phase of placebo use and at the follicular phase of OCP use. Thus, our results point at a different direction when compared to the results of previous investigations. The menstrual phase is the phase of the cycle during which conception is not possible. Moreover, independently of phase of the cycle, CL is absent when OCP is correctly used.⁵⁵ Thus, previous observations that higher ratings of female voice-related sexual attractiveness to males when CL is high^{9,57} are not corroborated by our results.

Taken our results, the question now is why female voice-related sexual attractiveness to males is higher when CL is low. Our explanation follows the line of thought that female voice-related sexual attractiveness to males possibly acts as a cue of female’s extended sexuality rather than of a biological reproductive one. According to recent evolution-minded theories of human reproduction, women possess a “dual sexuality,” ie, a sexuality for conception, using the fertile phase of their cycles, and a sexuality for other reasons, in the non-fertile phases of the cycle.⁵⁸ Concealing cues of different degrees of CL would increase male’s investment across the whole menstrual cycle and facilitate female’s extra-pair mating. Thus, males sexual attraction to females may be a consequence of what females do in order to regulate their extended sexuality across the menstrual cycle rather than estrus alone.⁵⁹ Following the same line of thought, the time window for conception during a menstrual cycle is short and the female’s detection of ovulation is difficult. From a purely biological perspective, women are

sexually active throughout the entire menstrual cycle also to increase their reproductive success.⁶⁰ In addition, from a sociological perspective of human reproductive behavior, one may consider that female sexual responsiveness is free from dependence upon an ovarian cycle. A variety of social situations may lead to completion of mating in any stage of the reproductive cycle.⁶¹ Therefore, one may ask why would females benefit from conveying fertility through voice?

The present findings are consistent with prior literature suggesting that the voice, compared to visual, behavioral and olfactory cues, is a much weaker predictor of sexual attractiveness^{59,62,63}. In this sense, although previous investigations have found that higher f_o has been related to sexier female bodies^{30,31} and to fertile phases of the cycle,⁴ these results should be interpreted with caution. First, one may argue that substantial differences in female voice-related sexual attractiveness to males would require great cycle-related variations in voice production. A recent study found that, although sf_o , sf_oSD and maximum f_o all vary between phases of the menstrual cycle,²⁰ only sf_oSD differences would fall above the 2 ST just noticeable interval required for these differences to be perceived in connected speech.⁶⁴ Moreover, controversial results still exist concerning the effects of sex steroid hormones on acoustical parameters of the voice. On the one hand some studies have found no differences in f_o between phases of the menstrual cycle.^{24,65,66} On the other hand, others have found a lowering of f_o during premenstrual phase as compared to ovulation^{4,67}. Second, not all studies agree that female voice-related sexual attractiveness to males is higher at ovulation and at the fertile phase of the menstrual cycle because these phases present higher f_o values. For example, Karthikeyan & Locke (2015) found that although men preferred voices at ovulation, this phase presented the lowest f_o values. In addition, although Puts *et al.*, (2013) found that males find female voices more attractive at the fertile phase of the menstrual cycle, a relationship between f_o and attractiveness was not found. Indeed, given the multimodal nature of female voice-related sexual attractiveness to males,⁶⁸ to attribute attractiveness to an isolated morphometric acoustical parameter of voice production such as f_o seems to be insufficient. Voice-related sexual attractiveness may well be associated with other acoustical features of the voice.⁶⁹ Finding an association between female voice-related sexual attractiveness to males and a voice parameter that reflects linguistic contents and emotions seems to fit best the underlying complexity of psychoacoustical models of voice perception.⁷⁰ For example, the variation of f_o in a sentence is an important attribute when communicating verbal and emotional meanings.^{71,72} However, no studies have investigated the df_o and/or the rate of f_o change when it concerns the perception of female voice-related sexual attractiveness to males. These parameters seem worthwhile to investigate, given that they reflect, for example linguistic information.⁷³ The present work found a medium-large effect of df_o on voices rated as more sexually attractive. The higher the df_o , the lower the probability of obtaining higher rankings of

attractiveness. In other words, males find female voices more sexually attractive when their f_o contour variations are smoother. Fundamental frequency contour variations reflect the interaction of pragmatic and paralinguistic functions, and these are learnt speech features according to cultural and social backgrounds.⁷³ Also, basic emotions have been discriminated through f_o contour variability.⁷²

It is worth mentioning that differences between our results and results of earlier investigations may well be explained by the substantial heterogeneity of study designs. Indeed, Wheatley *et al.* (2014) found completely different associations between female voice-related sexual attractiveness to males and concentrations of T depending on whether a within or a between-subject design was used.⁷⁴ The present study uses voices collected at different phases of the menstrual cycle and at different degrees of CL, collected in a previous RCT. Thus, contrary to primary investigations comparing voices of different women recorded at different phases of the cycle and at different CL conditions, voices collected during this RCT offered the possibility of within-subject ratings. This has been considered preferable when evaluating voice characteristics under different conditions for at least two reasons: (1) vocal behavior is highly idiosyncratic; and (2) voices are unique and might be affected in different ways depending on external factors.⁷⁵

Other limitations of previous studies include the absence of measurements of hormonal concentrations in serum across phases of the menstrual cycle. One might argue that the high variability of distributions of lengths of follicular and luteal phases may blur the results if measures of blood circulating hormones are absent.⁶³ Moreover, previous perceptual experiments lacked on the inclusion of randomly allocated repeated stimuli. This prevented understanding of consistency and reliability of the given female voice-related attractiveness to males.

The study design presented here seems to be a step forward in providing a methodological example for future perceptual experiments on female voice-related attractiveness to males. However, possible pitfalls should be discussed. First, all voices included in the perceptual experiment were singer's voices. The vocal technique learnt by these women could be reflected in their f_o range, thus creating a bias in the results. However, our data fell within sf_o values previously reported for normal, non-pathological voices considered as attractive³⁴ and belonging to an age-matched group of women (20 to 32 yrs.) who were not singers nor professional voice users.⁷⁶ Testing whether singers' speaking voices could be rated as more attractive than those of a non-singer was beyond the scope of this investigation. Second, one could argue that ratings of female voice-related attractiveness to males were difficult to make as the 6 stimuli presented in each Visor window were very similar. To try to circumvent this limitation, cognitive load of the listener was reduced with the selection of a short phrase⁷⁷. This would have accounted for the 6.7% and 7.3% rates of missing values found for runs A and B of the listening test, respectively. These rates are high when compared to the 2% found per

rater in other perceptual tests of female voice-related attractiveness to males.⁴³ However, proportionally to the total number of evaluations, our missing values yielded a 2.4% per rater. Second, the Cohen's kappa for the concordance of ratings of voice-related sexiness between runs A and B of the listening test was small. One may argue that such concordance is expected in within-subject comparisons of the same voice reading the same phrase 6 times. Moreover, the sensitivity analysis between runs A and B showed a similar result, thus increasing confidence of results.

Another possible pitfall could exist related to the fact that only 9 female voices were recorded, which would reflect a small sample size. However, this was not the case: for each of the 9 female recorded voices, 6 stimuli were sorted and rated per voice, which yielded a total of 54 stimuli judged per rater. Following Judd *et al.* (2012) simulations on the benefits of increasing the number of participants as compared to increasing the number of stimuli, the 54 stimuli per rater would result in a statistical power of 0.5 to 0.6 in a design such as the one here presented, where participants were crossed with condition, and stimuli were nested under two levels, ie, listener and female.⁷⁸

CONCLUSIONS

In the present study, perceptions of female voice-related attractiveness were explored for 78 heterosexual males using voice samples taken from 9 women recorded at three phases of the menstrual cycle and at different conditions, i.e., placebo and OCP use. These placebo and OCP conditions were randomly and double-blinded allocated in a RCT. Both voice parameters and concentrations of sex steroid hormones in blood were analyzed. The results of this experimental design seem to point at a different direction when compared to previous results: female voice-related attractiveness to males is high when CL is low or even absent, when both E₂/P and T concentrations are low and at low df_o values. Thus, it seems that female voice-related attractiveness to males may be a consequence of what females do in order to regulate their extended sexuality across the menstrual cycle.

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