

# Vocal Acoustic and Auditory-Perceptual Characteristics During Fluctuations in Estradiol Levels During the Menstrual Cycle: A Longitudinal Study

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**Summary:** Estradiol production varies cyclically, changes in levels are hypothesized to affect the voice. The main objective of this study was to investigate vocal acoustic and auditory-perceptual characteristics during fluctuations in the levels of the hormone estradiol during the menstrual cycle. A total of 44 volunteers aged between 18 and 45 were selected. Of these, 27 women with regular menstrual cycles comprised the test group (TG) and 17 combined oral contraceptive users comprised the control group (CG). The study was performed in two phases. In phase 1, anamnesis was performed. Subsequently, the TG underwent blood sample collection for measurement of estradiol levels and voice recording for later acoustic and auditory-perceptual analysis. The CG underwent only voice recording. Phase 2 involved the same measurements as phase 1 for each group. Variables were evaluated using descriptive and inferential analysis to compare groups and phases and to determine relationships between variables. Voice changes were found during the menstrual cycle, and such changes were determined to be related to variations in estradiol levels. Impaired voice quality was observed to be associated with decreased levels of estradiol. The CG did not demonstrate significant vocal changes during phases 1 and 2. The TG showed significant increases in vocal parameters of roughness, tension, and instability during phase 2 (the period of low estradiol levels) when compared with the CG. Low estradiol levels were also found to be negatively correlated with the parameters of tension, instability, and jitter and positively correlated with fundamental voice frequency.

**Key Words:** Menstrual cycle—Estradiol—Estrogen—Hormones—Voice.

## INTRODUCTION

Estrogen is a steroid hormone that acts on different systems via intracellular receptors.<sup>1,2</sup> In addition to its actions in the reproductive system, estrogen is also involved in neuroprotection, affects mood, and acts as a vasodilator that may have cardioprotective effects.<sup>2–5</sup>

Estradiol, estriol, and estrone make up the hormone estrogen.<sup>6</sup> Estradiol production varies during the menstrual cycle, with the highest serum concentrations occurring near the ovulatory period (late follicular phase) and the lowest concentrations occurring during the premenstrual period (late luteal phase).<sup>2</sup> Fluctuations in levels of progesterone and estrogen alter neurotransmitter levels, which consequently modify the motor and sensory processes involved in laryngeal control.<sup>7</sup>

A previous study showed that androgen, estrogen, and progesterone receptors are expressed in the nuclei and cytoplasm of vocal fold cells, suggesting that these hormones may also act peripherally on the voice.<sup>8</sup> However, a later study investigated the presence of these receptors in laryngeal tissue and could not confirm their expression.<sup>9</sup> Another study reported

that fluctuations in estrogen and progesterone levels during the menstrual cycle could cause changes in laryngeal tissue.<sup>10</sup>

Manifestations in the larynx, such as physiological, anatomical, and psychological changes secondary to endocrine changes, may be associated with premenstrual vocal syndrome.<sup>11</sup> Days before menstruation, approximately one-third of women experience vocal changes, which may be characterized by voice fatigue, decreased vocal frequency, and a loss of projection and certain harmonics.<sup>11</sup> Vocal characteristics during the premenstrual period are related to hormonal levels. A previous study found that the shimmer, jitter, and harmonic-to-noise ratio (HNR) parameters extracted from acoustic analysis exhibit higher values during the luteal phase than in the follicular and ovulatory phases, and the fundamental frequency ( $F_0$ ) parameter was significantly lower during the luteal phase. The authors believe that this finding is due to an increase in vocal fold mass.<sup>12</sup> By contrast, auditory-perceptual evaluation using the GRBAS scale and self-evaluation using the Voice Handicap Index protocol showed no significant results when comparing menstrual cycle phases.<sup>12</sup>

A previous study that evaluated  $F_0$  during the menstrual cycle found that the voice tended to become deeper during the late luteal phase, but no relationship was found between premenstrual syndrome (PMS) and a reduction in  $F_0$ .<sup>13</sup>

Previous studies have investigated aspects of the voice during specific phases of the menstrual cycle.<sup>12,14–17</sup> These studies evaluated voice parameters across menstrual cycle phases, but there is no consensus on the parameters that change during these phases. Furthermore, most of these studies did not use clinical procedures to identify the cycle phase being investigated.

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Studies have also been conducted on hormonal contraceptive use and voice and have shown greater vocal stability in oral contraceptive users.<sup>18,19</sup> Combined oral contraceptives, which are composed of estrogen and progesterone, are intended to maintain the levels of these hormones, thus suppressing the menstrual cycle.<sup>1</sup>

Estrogens, particularly estradiol, are responsible for many of the changes that occur at puberty and for secondary sexual characteristics, including the voice.<sup>20</sup> In the menstrual cycle, psychological and physiological changes are most obvious during the peak and decline of estrogen levels. Earlier studies, which had distinct methodological aspects compared with this article, reported loss of vocal quality in the days before menstruation and at the beginning of the menstrual cycle when the estrogen level is very low.<sup>11,17,21</sup> Therefore, we investigated the relationship between estradiol during these periods of high and low estrogen concentrations and voice parameters.

The considerations outlined above demonstrate the need for longitudinal studies with greater methodological rigor that more thoroughly investigate the action of estrogen on voice quality during the menstrual cycle. The results of this study may help to direct patients with vocal complaints toward a specific treatment, prevent voice disorders, and increase our knowledge of the effects of hormonal changes on the voice during the menstrual cycle. In particular, this study intends to inform voice professionals and offer greater refinement of vocal performance interventions.

The objectives of this study are to (1) investigate vocal acoustic and auditory-perceptual characteristics during estradiol-level fluctuations during the menstrual cycle; (2) measure estradiol levels during the late luteal and late follicular phases of the menstrual cycle; (3) compare the results obtained for the test group (TG) and the control group (CG); and (4) examine the relationship between estradiol and voice quality during the menstrual cycle.

## MATERIALS AND METHODS

### Ethical considerations

This study was approved by the Ethics Committee for Research with Human Beings of the Health Sciences Center of a higher education institution under protocol No. 0657/13.

Before the experiment, each participant was informed about the purpose and procedures of the study and signed a free and informed consent form in accordance with Resolution 466/12 of the National Research Ethics Commission (Comissão Nacional de Ética em Pesquisa). Each participant was oriented in relation to all procedures to be performed and was prompted for her permission to use her data in scientific research without having her identity revealed. Furthermore, each participant was informed of the possibility to withdraw her consent at any time.

### Study design

This is a longitudinal, observational, quantitative, explanatory field study.

### Sample

Initially, 62 women agreed to participate. Of these, 10 did not meet the eligibility criteria and six were excluded due to attending only the first session. Thus, 44 participants were selected from a convenience sample. The participants were between 18 and 45 years old, which are the average ages of the female reproductive life.<sup>22</sup> Of the participants, 27 women with a regular menstrual cycle formed the TG and 17 women who used combined oral contraceptives were allocated to the CG.

The following eligibility criteria were used when forming the study groups: individuals between 18 and 45 years of age; absence of neurological, hormonal, and ovarian diseases; absence of pregnancy and lactation within the previous 6 months; in the TG, a regular menstrual cycle; and no use of medications containing hormones, except for combined oral contraceptives in the case of the CG.

For the CG, in addition to the eligibility criteria mentioned above, the selected volunteers must have been taking single-phase, low-hormonal-dose combined oral contraceptives.

Below are the brands of oral contraceptives used by the participants of this study and their hormone concentrations:

Diane<sup>®</sup> 35, Schering do Brasil: 2 mg cyproterone acetate + 0.035 mg ethinylestradiol

Femina, Gedeon Richter Plc., Hungary, imported by Aché Laboratórios Farmacêuticos S.A.: 150 mcg desogestrel + 0.02 mg ethinylestradiol

Elani Ciclo, Libbs farmacêutica Ltda.: 3 mg drospirenone + 0.03 mg ethinylestradiol

Micropil<sup>®</sup>, EMS S/A: 0.075 mg gestodene + 0.03 mg ethinylestradiol

Microvlar<sup>®</sup>, Schering do Brasil: 0.15 mg levonorgestrel + 0.03 mg ethinylestradiol

Selene<sup>®</sup>, Eurofarma laboratórios S.A.: 2 mg cyproterone acetate + 0.035 mg ethinylestradiol

Yasmin<sup>®</sup>, Schering do Brasil: 3 mg drospirenone + 0.03 mg ethinylestradiol

### Measurements

Anamnesis, hormone level measurement, and voice sample collection were performed on each study participant for auditory-perceptual evaluation and acoustic analysis.

### Anamnesis

Anamnesis was conducted using a questionnaire prepared by the Group of Studies and Research into Hearing, Balance and Tinnitus (Grupo de Estudos e Pesquisa em Audição, Equilíbrio e Zumbido) and adapted for this study to characterize participants and apply eligibility criteria. Participants were interviewed and asked for personal information (age and profession), information regarding their menstrual cycle (cycle length, regularity, flow, and day of last menstruation), medical history (contraceptive use and type of contraceptive, use of hormonal medication or other

medication), and aspects related to overall health, including whether they had voice problems.

### Hormone levels

Levels of estradiol, the main and most powerful estrogen released from the ovaries,<sup>1</sup> were measured during the late follicular (close to the ovulatory period) and late luteal (premenstrual) phase to verify fluctuations in serum concentrations of this hormone during the menstrual cycle. Blood samples were always collected between 7:00 and 9:00 am, after the participant had fasted for at least 4 hours. Sessions were designated phases 1 and 2 to refer to high and low estradiol concentrations.

Chemiluminescence microparticle immunoassays were performed using an Abbot Architect I 2000 device to establish estradiol concentrations. Blood sample collection and hormone level measurements were performed in the Laboratory of Clinical Analysis of the Lauro Wanderley University Hospital (Hospital Universitário Lauro Wanderley) in the city of João Pessoa, Paraíba.

The reference values of estrogen by chemiluminescence microparticle immunoassays in the device from Abbott Architect are listed below:

Follicular phase: 21.0–251.0 pg/mL  
 Middle of the cycle: 38.0–649.0 pg/mL  
 Luteal phase: 21.0–312.0 pg/mL  
 Post menopause: <10–28.0 pg/mL  
 Source: Lauro Wanderley University Hospital/João Pessoa, Paraíba, Brazil.

The progesterone reference values by chemiluminescence immunoassay are listed below:

Follicular phase: <0.1–0.30 ng/mL  
 Luteal phase: 1.2–15.9 ng/mL  
 Post menopause: <0.1–0.2 ng/mL  
 Pregnant women  
 First trimester: 2.8–147.3 ng/mL  
 Second trimester: 22.5–95.3 ng/mL  
 Third trimester: 27.9–242.5 ng/mL  
 Source: Lauro Wanderley University Hospital/João Pessoa, Paraíba, Brazil.

### Voice parameters

The evaluated voice parameters were obtained using voice sample collection. A recording of the sustained vowel /*e*/ was made for later acoustic and auditory-perceptual voice quality analysis. Voice collection was performed in a sound-treated booth with ambient noise of less than 50 dB of sound pressure level (SPL) in the Laboratory of Integrated Voice Studies (Laboratório Integrado de Estudos da Voz), Department of Speech Therapy, Universidade Federal da Paraíba.

An E835 Sennheiser unidirectional cardioid microphone was used, which was fixed on a pedestal and coupled to a Dell desktop using a Behringer audio interface, model U-Phoria UMC 204, and FonoView software (CTS Informática).

The sampling rate used was 44,100 Hz, which preserves most voice signal information. During recording, the microphone was placed at an average distance of 10 cm from the labial commissure.

Voice samples were subjected to the following analyses: (1) acoustic: parameters relating to  $F_0$ , jitter, shimmer, and HNR were evaluated. The values of these parameters were extracted using *VoxMetria* software; (2) an auditory-perceptual evaluation was performed using a visual analogue scale (VAS) with a 100-mm horizontal line.

Traditional classifications were considered when interpreting this scale,<sup>23</sup> in which the normal variability of voice quality corresponds to 0–35.5 mm, a mild to moderate deviation in intensity corresponds to 35.6–50.5 mm, a moderate deviation is between 50.6 and 90.5 mm, and intense deviation is between 90.6 and 100 mm. The overall degrees (ODs) of vocal deviation, roughness, breathiness, tension, and vocal instability were evaluated.

Before beginning the auditory-perceptual evaluation, the judges studied the concepts underlying each voice parameter under investigation and listened to anchor stimuli with predominant and intense voice deviations to assist in the scoring of the voice parameters. The voice evaluation focused on the glottal source based on the sustained vowel /*e*/ speech task. Tension and instability, as well as all other parameters, were scored from 0 to 100. Phonatory tension was measured, which requires substantial muscular effort to be perceived. If the tension was altered, the judge was to note whether it was hypotensive or hypertensive; hypertension was unanimously reported by the judges. Therefore, in this study, when we refer to alteration, we are referring to hypertension. Vocal instability was evaluated based on the concept of noticeable irregular or regular variations in the  $F_0$ . With these concepts and with the anchor stimuli, we believe that the judges were able to perform the best possible auditory-perceptual evaluation.

Auditory-perceptual evaluation was performed by three judges, all of whom are speech therapists specializing in voice. Before beginning the evaluation, sustained /*e*/ vowel anchor stimuli for healthy voices and voices with mild, moderate, and intense vocal deviations were provided along with different possible predominances in voice quality. The judges reviewed 88 randomly matched voices, with 20% of the vocal samples repeated, to test the degree of reliability and the accuracy of intrajudge evaluations.

Two voice samples from the same subject were presented to the judges in a random order; that is, the judges were blinded to the phases (1 or 2). The judges were also unaware of which group each voice belonged to, so they were also blinded to the groups. Although each voice sample was presented to the three judges at the same time, each judge assessed the voice independently and confidentially.

Interjudge reliability was also evaluated. These analytical procedures were performed to ensure the confidence level of this vocal evaluation procedure. As interjudge agreement did not show significant differences, we used the responses of the judge with the best internal agreement. The selected

judge showed an excellent level of intrajudge agreement, with an intraclass correlation coefficient of 0.82.

## Procedures

This study occurred in two phases, as described below.

### Phase 1

Initially, an informed consent form was given to each subject and anamnesis was performed using a questionnaire. In this phase, the TG subjects were evaluated in the late follicular phase (11th–13th day of the menstrual cycle), which corresponds to the period when estradiol levels are elevated. Blood was collected from the TG group participants to determine their serum estradiol concentrations, and voice samples were collected shortly after (for acoustic and auditory-perceptual analyses).

The CG was evaluated 1 day after the ingestion of the 10th contraceptive pill, at which time hormone levels are stable.<sup>24,25</sup> The CG participants underwent voice collection only. Measurements were taken from all study participants at approximately the same time each day to control for the effects of circadian rhythm on serum estradiol concentrations.<sup>26</sup>

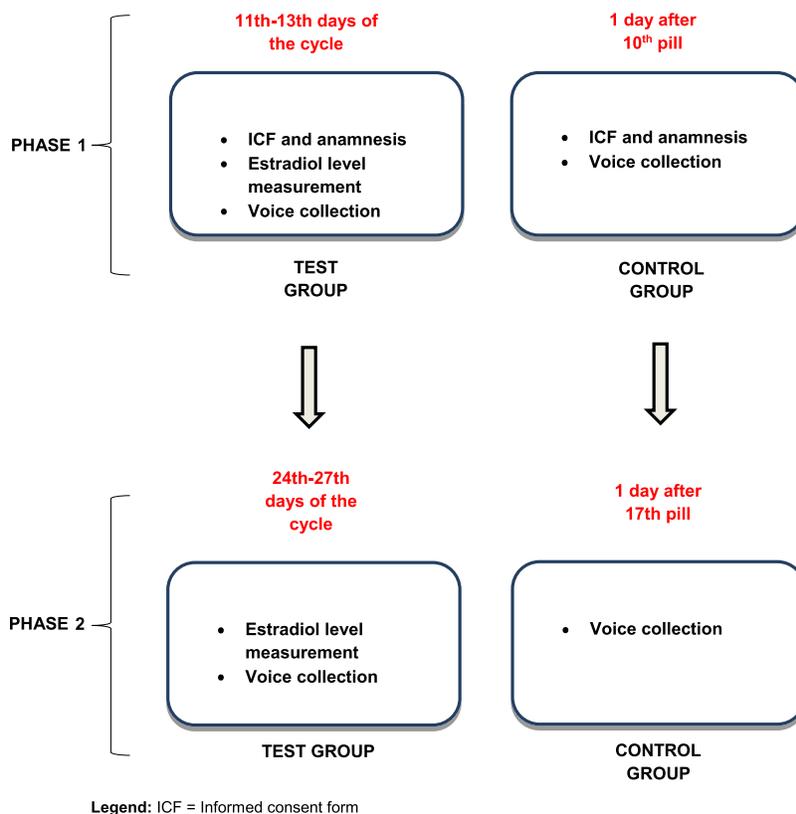
### Phase 2

The TG volunteers were tested during the late luteal phase (24th–27th day), which corresponds to the period when estradiol levels are low. As in phase 1, in this phase, the TG

participants underwent blood sampling to measure their estradiol concentrations and subsequently underwent voice collection. The CG was evaluated 1 day after ingesting the 17th pill, which is a period of stability for synthetic contraceptive hormones.<sup>24,25</sup> The measurements taken in this phase were the same as those taken in phase 1 for each group. Figure 1 shows a flowchart of the methodological procedures used in this study.

## DATA ANALYSIS

Data were categorized and entered into a digital spreadsheet. Statistical analyses were performed that were both inferential and descriptive, including frequency, mean, and standard deviation measurements. The Kolmogorov-Smirnov test was performed to determine whether the data followed a normal distribution. This allowed the following parametric tests to be performed: the Student's *t* test for independent samples, to compare means related to vocal characteristics between the case and control groups; the Student's *t* test for related samples, to compare intragroup vocal characteristics according to hormone levels; and Pearson's correlation test to correlate participants' vocal and hormonal data. The statistical software *R* version 2.11.0. was used, and the significance level was set to 5% (R Core Team [2016]. *R*: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org>).



**FIGURE 1.** Flowchart of the methodological procedures used in this study.

## RESULTS

The TG participants had a mean age of 27.37 ( $\pm 7.97$ ) and a mean menstrual cycle duration of 29.00 ( $\pm 2.34$ ) days. The participants allocated to the CG, who had used low-dose monophasic combined oral contraceptives for approximately 24.31 ( $\pm 21.26$ ) months, had a mean age of 23.18 ( $\pm 3.28$ ) years.

Table 1 shows the intragroup changes that occurred in certain parameters across the two test phases for the TG and the GC. For the TG, a significant difference was observed in estradiol levels between phases 1 and 2 ( $P = 0.0001$ ) and in the following auditory-perceptual voice parameters: OD ( $P = 0.041$ ), tension ( $P = 0.033$ ), and instability ( $P = 0.007$ ). No differences between phases were observed in the TG's acoustic evaluation. There was no significant difference in any of the vocal aspects evaluated in the CG when comparing phases 1 and 2.

The mean voice parameters that showed significant changes in the TG were as follows: OD, with a score of 45.31 ( $\pm 13.52$ ) in phase 1 and 49.52 ( $\pm 13.81$ ) in phase 2; tension, with a score of 32.93 ( $\pm 16.27$ ) in phase 1 and 37.83 ( $\pm 16.82$ ) in phase 2; and instability, with a score of 37.09 ( $\pm 14.40$ ) in phase 1 and 41.61 ( $\pm 15.53$ ) in phase 2.

A comparison of vocal aspects was performed between the TG and the CG in phases 1 (high estradiol levels) and 2 (low estradiol levels). The groups differed in the degree of roughness ( $P = 0.037$ ) in phase 1 and the degree of roughness ( $P = 0.025$ ), tension ( $P = 0.001$ ), and instability ( $P = 0.039$ ) in phase 2. All of these parameters exhibited the highest degree of vocal deviation in the TG, as shown in Table 2.

Table 3 shows data pertaining to the correlation between the TG participants' estradiol levels and voice parameters in the two phases studied. There was a negative correlation between hormone levels in phase 1 and the vocal aspects of tension ( $P = 0.042$ ), instability ( $P = 0.046$ ), and jitter in phase 2 and a positive correlation between estradiol level and  $F_0$  ( $P = 0.049$ ), both in phase 2.

## DISCUSSION

This study found voice fluctuations during the menstrual cycle and that combined oral contraceptive use is related to the stability of voice quality. When comparing phases 1 and 2 within the participant groups, a significant increase in vocal deviation in the TG was observed during phase 2, when estradiol levels were low. The auditory-perceptual analysis of voice recordings from this group also revealed that voices were significantly more unstable and tense during this phase. Tension had normal variability in phase 1 but underwent a deviation of mild intensity in phase 2. There was no significant change in the auditory-perceptual evaluation in the CG, and changes in acoustic analysis parameters were not sensitive enough to reveal significant voice changes in either group across the evaluated phases.

A previous study mapping vocal characteristics across menstrual cycle phases revealed considerable differences in the degree of dysphonia, roughness, breathiness, asthenia, and tension between the cycle phases studied. During phases in which estradiol levels were higher, voice quality was

**TABLE 1.**  
Intragroup Comparison of Hormonal and Vocal Acoustic and Auditory-Perceptual Parameters During the Menstrual Cycle

Variable	Phase 1		Phase 2		P Value	
	Mean	SD	Mean	SD		
Test group	Estradiol	187.52	115.80	99.11	80.90	0.0001*
	$F_0$ (Hz)	206.56	18.50	204.32	22.20	0.320
	Jitter	0.334	0.544	0.570	1.682	0.497
	Shimmer	2.547	1.200	3.510	6.662	0.372
	HNR	0.833	0.125	0.830	0.122	0.911
	VAS—OD	45.31	13.52	49.52	13.81	0.041*
	VAS—DR	35.39	15.13	37.80	17.70	0.342
	VAS—DB	26.75	19.91	25.74	18.63	0.709
	VAS—DT	32.93	16.27	37.83	16.82	0.033*
	VAS—DI	37.09	14.40	41.61	15.53	0.007*
Control group	$F_0$ (Hz)	210.93	22.05	213.71	21.12	0.367
	Jitter	0.293	0.306	0.210	0.066	0.275
	Shimmer	2.370	1.010	1.954	0.425	0.086
	HNR	0.824	0.144	0.820	0.133	0.870
	VAS—OD	42.32	14.34	41.71	8.27	0.850
	VAS—DR	25.15	15.69	26.00	13.84	0.756
	VAS—DB	24.25	21.41	26.88	17.71	0.497
	VAS—DT	24.76	17.16	20.82	14.01	0.198
	VAS—DI	33.29	14.60	33.29	10.31	0.764

Source: João Pessoa, 2017.

\*  $P < 0.05$ .

Student's *t* test for paired data.

Abbreviations: DB, degree of breathiness; DI, degree of instability; DR, degree of roughness; DT, degree of tension; SD, standard deviation.

**TABLE 2.**  
**Comparison of Women's Vocal Acoustic and Auditory-Perceptual Parameters Between the Test and Control Groups**

Variable	Test Group		Control Group		P Value	
	Mean	SD	Mean	SD		
Phase 1	F <sub>0</sub> (Hz)	206.56	18.50	210.93	22.05	0.482
	Jitter	0.334	0.544	0.293	0.306	0.779
	Shimmer	2.547	1.200	2.370	1.010	0.624
	HNR	0.833	0.125	0.824	0.144	0.820
	VAS—OD	45.31	13.52	42.32	14.34	0.490
	VAS—DR	35.39	15.13	25.15	15.69	0.037*
	VAS—DB	26.75	19.91	24.25	21.41	0.683
	VAS—DT	32.93	16.27	24.76	17.16	0.120
	VAS—DI	37.09	14.40	33.29	14.60	0.312
Phase 2	F <sub>0</sub> (Hz)	204.32	22.20	213.71	21.12	0.171
	Jitter	0.570	1.682	0.210	0.066	0.387
	Shimmer	3.510	6.662	1.954	0.425	0.268
	HNR	0.830	0.122	0.820	0.133	0.786
	VAS—OD	49.52	13.81	41.71	8.27	0.125
	VAS—DR	37.80	17.70	26.00	13.84	0.025*
	VAS—DB	25.74	18.63	26.88	17.71	0.841
	VAS—DT	37.83	16.82	20.82	14.01	0.001*
	VAS—DI	41.61	15.53	33.29	10.31	0.039*

Source: João Pessoa, 2017.

\*  $P < 0.05$ .

Abbreviations: DB, degree of breathiness; DI, degree of instability; DR, degree of roughness; DT, degree of tension; SD, standard deviation. Student's *t* test— independent samples.

**TABLE 3.**  
**Correlations Between Estradiol Level and Vocal Acoustic and Auditory-Perceptual Parameters in Test Group Participants Evaluated During Two Phases**

Variable	Phase 1		Phase 2	
	Test Statistic	P Value	Test Statistic	P Value
Estradiol phase 1				
F <sub>0</sub> (Hz)	0.015	0.941	-0.079	0.694
Jitter	0.137	0.497	-0.165	0.0412*
Shimmer	-0.028	0.892	-0.186	0.354
HNR	0.125	0.535	0.166	0.407
VAS—OD	-0.147	0.465	-0.248	0.213
VAS—DR	0.043	0.883	-0.051	0.801
VAS—DB	-0.224	0.262	-0.184	0.358
VAS—DT	-0.038	0.851	-0.378	0.042*
VAS—DI	-0.212	0.287	-0.337	0.046*
Estradiol phase 2				
F <sub>0</sub> (Hz)	0.316	0.109	0.383	0.049*
Jitter	0.230	0.249	-0.094	0.640
Shimmer	-0.046	0.819	-0.130	0.518
HNR	0.350	0.073	0.224	0.260
VAS—OD	0.002	0.992	-0.149	0.458
VAS—DR	-0.059	0.770	-0.227	0.255
VAS—DB	-0.191	0.340	-0.123	0.542
VAS—DT	0.066	0.743	-0.314	0.110
VAS—DI	-0.086	0.671	-0.173	0.388

Source: João Pessoa, 2017.

\*  $P < 0.05$ .

Pearson's correlation test.

Abbreviations: DB, degree of breathiness; DI, degree of instability; DR, degree of roughness; DT, degree of tension; SD, standard deviation.

improved when compared with phases in which levels of estradiol were lower,<sup>17</sup> and the same finding was confirmed in this study. There was a significant difference between estradiol levels in phases 1 and 2 in the TG and in the parameters of OD, tension, and instability measured by the auditory-perceptual evaluation. These parameters worsened in phase 2, demonstrating that variations in the parameters of OD, tension, and vocal instability accompanied changes in estradiol concentration.

The results of this study corroborate the literature; a previous Italian study investigated dysphonia during the menstrual cycle and showed fluctuations in voice quality in the early follicular and late luteal phases, when estradiol levels are reduced.<sup>21</sup> Research on vocal behavior during the menstrual cycle has shown changes in voice quality during the early follicular phase. In this phase, voices were more rough-breathy and unstable.<sup>15</sup> Vocal fold edema, which is common in women in this phase of the menstrual cycle, can cause vibratory irregularities, incomplete glottal closure (increased physiological posterior glottal gap), and muscle instability in the phonation system, which may result in the presence of a voice disorder marked by transient roughness, breathiness, and instability to varying degrees depending on the individual's predisposition.

Edema of the vocal folds, accompanied by thick mucus and loss of elasticity, can result in muscle function and vibratory cycle disturbance,<sup>11</sup> which may lead to transient voice alteration marked by roughness, breathiness, and instability varying in degree depending on individual predisposition.

No significant changes between phases were observed in the acoustic parameters of the TG. These findings agree with those of a study on voice changes during menstrual cycle phases, which measured estradiol concentration in each phase and found no significant changes in the acoustic parameters  $F_0$ , jitter, shimmer, and HNR.<sup>17</sup> These are the same parameters evaluated in this study, which are believed to be very sensitive. A great deal of variability (standard deviation) was found in the studied sample. To observe these changes at a significant level, a larger sample size would be necessary. However, this is difficult in research that investigates hormone levels, due to the need to control blood sample collection time and individual circadian cycles, and in terms of the funding necessary to enable hormonal analysis research.

In the CG, which was composed of oral contraceptive users, there were no significant differences in any vocal characteristics between phases; ie, voice quality was stable in both evaluated phases. These data were expected, as it is known that combined oral contraceptives facilitate the maintenance of constant estrogen and progesterone levels,<sup>1</sup> and the literature demonstrates greater voice stability in oral contraceptive users.<sup>18,19,27</sup> Studies<sup>18,19</sup> have performed acoustic evaluations of both users and nonusers of oral contraceptives, and the results of these studies suggest greater vocal stability in groups using contraceptives. One such study found that drospirenone, found in the combined oral contraceptive Yasmin, has properties that are able to

significantly reduce irregularities in the vibration pattern of the vocal folds during singers' performances.<sup>27</sup>

The extracted acoustic parameters and auditory-perceptual evaluations were also compared between the TG and the CG. The TG showed greater roughness in phase 1 and greater roughness, tension, and instability in phase 2 than the CG. This finding suggests variation in vocal aspects related to estradiol levels, as hormone levels fluctuated in the TG and were stable in the CG due to the use of a combined oral contraceptive.

A study on voice changes during the menstrual cycle evaluated women of reproductive age (experimental group) and postmenopausal women (control group) and reported that in the experimental group, in which estrogen levels were higher, better voice quality was found.<sup>28</sup> These findings are in accordance with those obtained in the present study.

Despite changes in the parameters of roughness, tension, and instability, no significant changes were observed in the OD of vocal deviation in an intergroup comparison. This could be explained by the mild voice changes observed during estradiol reduction.

Similar to the intragroup analysis mentioned above, no significant changes were found in an intergroup comparison of acoustic analysis. These results were similar to those of studies investigating the acoustic parameters of jitter, shimmer, and  $F_0$  in women of reproductive age and in menopause, which found no significant changes in these acoustic parameters between groups.<sup>29,30</sup>

A test was conducted to correlate estradiol levels with voice parameters, and a negative correlation was found between estradiol levels and jitter, degree of tension, and instability in phase 1, and a positive correlation between estradiol levels and  $F_0$  in phase 2. This reveals that the lower the serum estradiol concentration, the greater the jitter and mean tension and instability on the VAS, and the lower the estradiol level, the lower the  $F_0$  in the same phase.

The literature shows a relationship between voice and PMS, and increased jitter was found in a positive PMS group during the menstrual cycle phase with low estradiol levels (the late luteal phase).<sup>14</sup> A recent study found that during the menstrual phase in which estrogen levels are lower,  $F_0$  was significantly lower and HNR, jitter, and shimmer were higher; these values could be related to the increased mass of the vocal folds,<sup>12</sup> which affects changes in the frequency and amplitude of the glottal cycles. Another study using acoustic analysis found decreased  $F_0$  and changes in jitter, shimmer, and HNR during the menstrual cycle phase with low serum estradiol.<sup>16</sup> The  $F_0$  and jitter values obtained in this study corroborate the results of previous studies. Physiologically, the vocal folds may be heavier due to the edema inherent during this phase of the menstrual cycle, which affects the glottal cycle frequency (jitter) of the vocal folds.

In phase 2, when estrogen levels are lower, it is common to find changes in the larynx, such as edema of the vocal folds, with thick mucus, microvarices, loss of elasticity, and impaired muscle function and vibration cycle.<sup>11</sup> A study of premenstrual vocal syndrome revealed that the most common

symptoms of this syndrome are vocal fatigue, decreased  $F_0$ , dynamic field restriction, and loss of voice quality.<sup>11</sup>

Most studies on voice and the menstrual cycle base their measurements on cycle phases established using subjective procedures and do not measure estrogen levels during the investigated phases.<sup>12–16,28,31</sup> This may explain the lack of consensus regarding changes to auditory-perceptual and acoustic parameters during the menstrual cycle. The results of the present study suggest that vocal changes occur during the menstrual cycle and that such changes are related to variations in estradiol levels. For the TG, the auditory-perceptual evaluation demonstrated a significantly higher OD of vocal deviation, tension, and instability in phase 2, when serum estradiol levels are lower. The CG showed no significant changes in auditory-perceptual parameters between the two phases, which suggests that the hormonal stability promoted by contraceptives may facilitate the preservation of voice quality. Furthermore, decreases in estradiol levels correlated with increased tension, instability, and jitter and a reduction in  $F_0$ . The changes in these parameters may be explained by physiological changes in the larynx associated with reduced estradiol levels.

This study's findings also demonstrate the protective role of estrogen in the female body, adding the positive relationship between this hormone and the voice to its other actions already known to be beneficial, such as its role in reducing intraocular pressure, its assistance in the prevention and treatment of osteoporosis, and its cardioprotective and neuroprotective actions, including its actions on mood, memory, and cognition.<sup>2,5,32,33</sup>

The present study measured the predominant component of estrogen, estradiol, and found that changes in voice quality were related to changes in the levels of this hormone. Voice measurements were performed using acoustic and auditory-perceptual evaluations; the former are the most often cited in research on this subject, and the latter are more traditional, simple, and effective and are widely used in clinical practice.<sup>34,35</sup> Auditory-perceptual voice evaluation involves a subjective measurement but may be made more systematic and reliable by the use of scales and auditory-perceptual training using previous anchor stimuli for the analysis of voices of interest.<sup>36–38</sup> All of these procedures were followed when analyzing the 88 voice samples in this study to ensure our analysis was as accurate as possible.

The main limitations of the study were as follows: the small sample size, which was because this was a longitudinal study and, in particular, one that contained an invasive procedure (measurement of serum estradiol levels), and the absence of an anatomical and physiological laryngeal evaluation. Future research should be conducted on the subject following similar methodological procedures; however, laryngeal evaluation methods and a larger sample size should be included, if possible, to overcome these limitations.

This study may contribute to the understanding of the action of female hormones, especially estradiol, on the voice during the menstrual cycle. The results of this study show subtle changes in voice parameters between the studied phases,

which are hypothesized to be of great importance for speech therapy and may alert voice professionals to necessary interventions. The goal of these professionals is to achieve greater laryngeal effort, and it is therefore essential to know about changes to this organ, such as the physiological changes that occur during the menstrual cycle and consequently, in the voice. This knowledge will assist the professional in preserving voice quality independent of the menstrual cycle phase, thereby avoiding possible vocal disorders.

## CONCLUSIONS

Voice changes occur during the menstrual cycle, and these changes are related to fluctuations in estradiol levels. Decreasing levels of estradiol were associated with greater impairment in voice quality, increased vocal deviation, tension, and instability. The TG showed significant increases in roughness, tension, and instability in phase 2 when compared with the CG. The CG participants showed no significant voice changes between the investigated phases. Low estradiol levels were correlated with increased tension, instability, and jitter, as well as reduced  $F_0$ . Moreover, the beneficial action of estradiol on the voice underlines the protective role of estrogen in the female body.

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