The Effect of *In Vitro* Fertilization Treatment on Women's Voice

*Ofer Amir, *Natalie Lebi-Jacob, and †Ofer Harari, *Tel Aviv and †Haifa, Israel

Summary: Objectives. To examine the effect of *in vitro* fertilization (IVF) treatment on acoustic properties of women's voice.

Study Design. Prospective case series.

Methods. Ten women undergoing IVF treatment participated in the study. All participants were recorded repeatedly in three successive sessions, before and during treatment, in addition to collecting hormonal assays, endometrial thickness measurements, and follicular growth data. Recordings were performed while sustaining an isolated vowel repeatedly and during a reading task. Acoustic analyses included a limited set of fundamental frequency (F_0) measures, as well as frequency- and amplitude perturbation measures. Repeated-measure analyses of variance were performed to test for the treatment effect, and the correlations between the acoustic measures and the hormonal as well as endometrial thickness data were examined.

Results. A significant reduction in the two F_0 measures and in the amplitude perturbation measure was found throughout the treatment (P < 0.05). Before treatment, a negative correlation was found between F_0 and estrogen levels. After treatment, however, a negative correlation was found between F_0 and endometrial thickness.

Conclusion. An association was found between IVF treatment and specific voice properties. In addition, the possibility of a ceiling effect for the influence of estrogens on female vocal folds was introduced.

Key Words: IVF–Voice–Hormones–Vocal folds–Acoustic analysis.

INTRODUCTION

The human vocal folds, the larynx, and the entire voice mechanism are affected by the female hormonal system.¹ This effect has been confirmed by comparing cytological smears of the vocal folds' epithelium with cervical smears² and by the discovery of hormonal receptors in the larvngeal mucosa and epithelium.^{1,3} Behavioral support for this relationship was found in various conditions, and acoustic evidence has been reported for a relationship between specific vocal measures and the cyclic fluctuations in sex hormone levels throughout the reproductive years.⁴ Vocal changes associated with the decrease in hormonal levels during menopause have been reported, as well as a contradictory effect of hormonal replacement therapy on voice at this phase.^{4,5} During pregnancy, vocal changes were documented, which were associated with the sharp rise in hormonal levels and with the cessation of cyclic fluctuations.^{6,7} Furthermore, birth control pills were shown to affect acoustic properties of voice, as a function of the controlled and stable hormonal balance.^{8,9} Nonetheless, despite the growing body of research documenting the apparent relationship between the female hormonal system and the voice mechanism, these reported vocal changes have often been subclinical, inconsistent, or controversial.4,8,10

Women undergoing *in vitro* fertilization (IVF) treatment are exposed to substantially higher levels of estrogen,^{11,12} compared with women experiencing natural hormonal cycles¹³ or those using birth control pills.^{14,15} Therefore, our hypothesis

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was that a pronounced voice effect would be found in women undergoing IVF, more than in previously examined conditions. It is noteworthy, that a single recent study has entertained this possibility, using a subjective self-report approach, and did not find changes in *subjective* vocal symptoms (eg, vocal fatigue, vocal straining, and hoarseness) during the ovarian stimulation stage of IVF.¹⁶ Because of the inherent limitations and limited validity of the subjective self-evaluation of voice quality, we designed this study to examine possible acoustic changes in voice characteristics among women undergoing IVF treatment.

MATERIALS AND METHODS

Participants

This was a prospective study, conducted in a fertility clinic in Haifa and in the Department of Communication Disorders in Tel Aviv University, after obtaining the approval of the Tel-Aviv University Institutional Review Board (13-02-2011) and a written informed consent from all participants. Ten women (mean age: 31.1 years, range, 25–45), who enrolled for IVF treatment, volunteered to participate and completed a preliminary anamnesis questionnaire.

Speech and voice disorders were ruled out based on an assessment performed by two experienced speech-language pathologists, in addition to the participants' self-report. Exclusion criteria included a history of formal singing or voice training, voice or speech therapy, hearing loss, smoking, routine alcohol consumption, or substance abuse. All women were healthy, with no remarkable medical history and no routine medication.

Study protocol

All women were evaluated three times within the ovarian stimulation phase of the IVF treatment. *Session I* was performed before ovarian stimulation, before the beginning of the treatment. At this session, the participants' voice was recorded and blood tests were taken to obtain hormonal measurements.

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From the *Department of Communication Disorders, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; and the †Fertility and IVF Clinic, Haifa, Israel.

Address correspondence and reprint requests to Ofer Amir, Department of Communication Disorders, Sheba Medical Center, Tel Aviv University, Tel-Hashomer, 52621, Israel. E-mail: oferamir@post.tau.ac.il

| Session | Estrogen (pmol/L) | Progesterone (nmol/L) | LH (IU/L) | Endometrial thickness (mm) | VHI-10-Heb | | |
|---------|-------------------|-----------------------|-----------|----------------------------|-------------|--|--|
| 1 | 313.1 (247.3) | 4.9 (8.2) | 6.1 (3.2) | N/A | 4.00 (4.03) | | |
| 2 | 3171.3 (2713.3) | 2.1 (2.1) | 4.7 (3.9) | 8.7 (1.3) | 4.40 (4.35) | | |
| 3 | 7464.3 (5955.6) | 1.8 (1.0) | 4.7 (3.7) | 10.4 (1.0) | 4.10 (4.31) | | |

TABLE 1. Mean Values and Standard Deviations (in Parentheses) of Hormonal and Endometrial Thickness Data, as well as VHI-10-Heb Scores at the Three Recording Sessions

Abbreviation: N/A, not available.

Session II was performed on the average, on day 5 of the ovarian stimulation. During this session, participants were recorded, blood tests were taken, and sonographic examination was performed vaginally to document endometrial thickness and evaluate number and size of follicles. *Session III* was performed, on the average, 3 days after session II, based on follicle size measurement, aiming for a follicle size of approximately 18–19 mm. During this session, participants were, again, recorded; blood tests were taken; and vaginal sonographic examination was performed. The three sessions were performed over a period of 9 days, on the average (standard deviation = 3). In addition, all participants completed the Hebrew version of the Voice Handicap Index-10 (VHI-Heb-10)¹⁷ before each session.

Women presenting for IVF treatment in the participating clinic underwent a short downregulation protocol.¹⁸ Gonadotropinreleasing hormone agonist (Decapeptyl [Ferring] 0.1 mg) was injected daily from the first or second day of menstruation. Controlled ovarian hyperstimulation began, on the average, 4 days later, using daily FSH (Follicle-stimulating hormone) or FSH + LH (Luteinizing hormone) injections.

Voice recordings and acoustic analysis

Voice recordings were performed in a quiet room at the fertility clinic, during routine visits. To reduce a possible bias effect, recordings were performed by an investigator who was not informed about the examined acoustic measures. Participants were recorded while sustaining the vowel /a/ for 3 seconds, eight times repeatedly. In addition, participants were recorded while reading aloud the second paragraph of the "Thousand Islands" reading passage.¹⁹ To reduce order effect, four vowel repetitions were recorded before the recording of the passage, and the remaining four repetitions were recorded after the passage.

A Sennhieser PC20 headset-microphone (Sennhieser Communications GmbH, Wedemark, Germany) was used for recordings. The microphone was placed at a fixed distance of approximately 7 cm from the corner of the participant's mouth. Recordings were performed digitally, using *Goldwave* software, V5.58 (Goldwave, Inc., Newfoundland, Canada), with a sampling rate of 48 kHz (16 bit). Computerized acoustic analyses were performed using *Praat*, V.5.3.39 (Boersma & Weenick, University of Amsterdam, Amsterdam, The Netherlands),²⁰ and a basic set of six acoustic measures was obtained. These measures were selected as representing commonly used acoustic features of voice in association with hormonal fluctuations, as described previously.^{8,9,21} These measures included (1) two derivates of fundamental frequency (F_0) —(a) Mean F_0 and (b) Fundamental Frequency Range $(F_0$ -range); (2) Relative Average Frequency Perturbation with a five-cycle smoothing factor (RAP); (3) Amplitude Perturbation Quotient with an 11-cycle smoothing factor (APQ); (4) Noise-to-Harmonic Ratio (NHR); and (5) Autocorrelation.

Statistical analysis

All statistical analyses were performed using *SAS* v9.2 (SAS Institute Inc., Cary, NC), with a significance level set at 5%. When *post hoc* analyses were conducted, an adjusted *P* value was applied to correct for multiple comparisons. Statistical analyses were conducted separately for the vowel and reading tasks, and independent analyses of variance (ANOVA) with repeated-measures were conducted for each acoustical parameter. In addition, linear correlations were calculated between results of the acoustic measures and the hormonal and endometrial thickness data.

RESULTS

Group means of hormonal and endometrial thickness data, as well VHI-Heb-10 values are presented in Table 1. Note that no endometrial thickness data were available for session I since women were menstruating at that time. Inspection of these data show that VHI-Heb-10 values did not differ markedly between the three sessions. Accordingly, an ANOVA with repeated measure did not reveal a statistically significant difference for this measure ($F_{2,18} = 0.72$, P = 0.93). As shown, by session III, all participants met the criteria for inducing ovulation.²² Monitor of controlled ovarian hyperstimulation with menotrophines was based on estradiol blood levels and follicles' size. The criterion for inducing ovulation was follicular size of more than 18 mm of the main follicle.

Table 2 presents group means for all acoustic measures obtained from the participants' recordings during both vowel and reading tasks. Independent ANOVAs were performed for each acoustic measure to test for differences between sessions throughout the hormonal treatment.

In the vowel task, a significant main effect for treatment was found for F_0 -range and APQ [($F_{2,9} = 3.69$, P = 0.04) and ($F_{2,9} = 4.15$, P < 0.05), respectively]. Contrast analyses revealed a significant reduction in both measures (adjusted P < 0.05) from session I to session III but not between adjacent sessions (ie, sessions I and II or sessions II and III). Similarly, a consistent reduction in values of F_0 was observed, as treatment progressed. However, this reduction failed to reach statistical

TABLE 2.

| Mean Values and Standard Deviations (in Parentheses) of Acoustic Measures Obtained for the Sustained Vowel /a/ and | nd |
|--|----|
| Reading Passage at the Three Recording Sessions | |

| | | Vowel /a/ | | | Reading | | |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|--|
| Acoustic Measure | Session 1 | Session 2 | Session 3 | Session 1 | Session 2 | Session 3 | |
| <i>F</i> ₀ (Hz)* | 192.61 (18.27) | 191.01 (20.10) | 189.32 (19.31) | 197.37 (19.38) | 192.94 (18.81) | 190.31 (19.20) | |
| F_0 -range (Hz) | 13.89 (20.51) | 9.77 (17.13) | 7.61 (13.59) | 146.28 (39.93) | 140.28 (40.30) | 139.16 (48.44) | |
| RAP (%) | 0.24 (0.17) | 0.23 (0.32) | 0.20 (0.14) | 0.69 (0.23) | 0.71 (0.18) | 0.70 (0.16) | |
| APQ (%)† | 1.78 (0.90) | 1.77 (0.87) | 1.47 (0.48) | 5.98 (1.53) | 6.11 (1.48) | 6.33 (1.46) | |
| NHR | 0.007 (0.011) | 0.006 (0.010) | 0.005 (0.004) | 0.064 (0.022) | 0.599 (0.016) | 0.063 (0.021) | |
| Autocorrelation | 0.993 (0.007) | 0.993 (0.009) | 0.995 (0.003) | 0.953 (0.014) | 0.956 (0.010) | 0.953 (0.013) | |

* Significant treatment effect for the reading task (P < 0.05).

 † Significant treatment effect for the vowel task (P < 0.05).

significance ($F_{2,9} = 0.20$, P = 0.81). The other measures did not reveal a consistent change associated with treatment progress [RAP: ($F_{2,9} = 0.59$, P = 0.57); NHR: ($F_{2,9} = 0.57$, P = 0.58); and Autocorrelation: ($F_{2,9} = 0.54$, P = 0.59)].

In the reading task, a significant main effect for the treatment was found for F_0 ($F_{2,9} = 5.28$, P < 0.05). Contrast analysis revealed a significant reduction in F_0 (adjusted P < 0.05) from session I to session III but not between adjacent sessions. Similar to the results of F_0 , a consistent reduction was observed for F_0 -range, as treatment progressed. This difference, however, failed to reach statistical significance ($F_{2,9} = 2.13$, P = 0.17). All other measures did not reveal a consistent change associated with treatment progression [RAP: ($F_{2,9} = 0.05$, P = 0.94); NHR: ($F_{2,9} = 0.51$, P = 0.61); and Autocorrelation: ($F_{2,9} = 0.62$, P = 0.56)].

Further analysis was performed by assigning the 10 participants to two groups, based on final endometrial thickness measured at session III, as a general predictor of treatment success. Four women were assigned to a "Medium Endometrial" subgroup, which was defined as $\leq 10 \text{ mm}$ (mean 9.5 ± 0.70). The other six women were assigned to a "Thick Endometrial" subgroup, which was defined as >10 mm (mean 11.08 ± 0.73). Initial inspection of the data revealed higher values of the F_0 -related measures in the "Medium Endometrial" subgroup, compared with the "Thick Endometrial" subgroup. These values are presented in Table 3.

Separate ANOVAs with repeated-measures were conducted for the two measures, to test for group differences. A significant main effect for endometrial thickness category was found only for mean F_0 ($F_{1,8} = 8.82$, P = 0.018). In addition, this measure yielded a significant difference between sessions ($F_{1,8} = 8.68$, P = 0.019), but no significant session \times group interaction was found ($F_{1,8} = 1.71$, P = 0.227). No significant differences were found for F_0 -range. Figure 1 illustrates group differences between the Medium and Thick endometrial groups for F_0 .

Finally, during session I, statistically significant *negative* linear correlations were found, between estrogen levels and F_0 , in both vowel task (r = -0.68, P = 0.03) and in reading task (r = -0.63, P = 0.04). No significant correlations were found between endometrial thickness and any of the acoustic measures in this session.

During session II, no significant correlations were found between estrogen levels or endometrial thickness and any of the acoustic measures.

During session III, no significant correlations were found between estrogen levels and any of the acoustic measures. However, statistically significant *negative* correlations were found between endometrial thickness and F_0 , in both vowel task (r = -0.64, P = 0.04) and in reading task (r = -0.8, P = 0.004).

DISCUSSION

Our study presents a preliminary examination of the effect of IVF treatment on women's voice, combining acoustic analysis of voice with hormonal assays and endometrial thickness measurements. In contrast to a single previous *subjective*

TABLE 3.

| Mean Values and Standard Deviations (in Parentheses) of <i>F</i> 0-Related Measures in the Medium and Thick Endometrial |
|---|
| Groups for Vowel and Reading Tasks in the Three Recording Sessions |

| | Acoustic | Medium Endometrial | | Thick Endometrial Group | | | |
|---------|--|---------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Task | Measure | Session 1 | Session 2 | Session 3 | Session 1 | Session 2 | Session 3 |
| Vowel | F ₀ (Hz)* Forrange (Hz) | 206.50 (14.84) | 202.48 (23.99) | 196.32 (14.99) 9 76 (7 85) | 183.35 (14.02) 8 56 (4 30) | 183.65 (21.91) 6 10 (2 56) | 184.65 (21.91) |
| Reading | F ₀ (Hz)* F ₀ -range (Hz) | 215.37 (7.33) 164.07 (10.26) | 207.89 (13.78) 162.03 (26.59) | 204.18 (13.38) 145.92 (34.65) | 185.37 (15.17) 134.41 (24.01) | 182.98 (14.64) 125.79 (11.66) | 181.06 (17.55) 134.65 (22.91) |

* Significant main effect for endometrial thickness group (P < 0.05).



FIGURE 1. Mean F_0 values ± 1 standard error bars of the medium and thick endometrial groups obtained in the three recording sessions, during the reading task.

report,¹⁶ the present study reveals an association between hormonal changes induced by IVF treatment and specific voice characteristics. This association was evident by two major findings. First, as treatment progressed and estrogen levels were raised, women exhibited a reduction in F_0 , F_0 -range, and APQ values. Second, when women were under a natural low hormonal influence (ie, session I), significant correlations were found between F_0 and estrogen levels. However, when estrogen levels peaked (ie, session III), significant correlations were found between F_0 and endometrial thickness.

Estrogens have a hypertrophic and proliferative effect on mucosa, in addition to increasing capillary permeability.¹ These effects were shown in female vocal folds, similar to other target organs. Therefore, an increase in estrogen levels is expected to lead to an increase in vocal folds' mass due to edema and to a lowering in F_0 .^{23,24} This hypothesis was supported by our findings, showing a lowering in F_0 , as estrogen levels increased along the IVF treatment. This is in keeping with previous studies that reported a similar reduction in F_0 measures around ovulation, when estrogen levels are high, in women with natural hormonal cycles.^{4,10,25}

In addition to the lowering in F_0 , edematous vocal folds typically show an irregular vibratory pattern.²⁶ This disturbance in vibration can be quantified acoustically by elevated values of frequency- and/or amplitude perturbation measures (eg, RAP and APQ, respectively). Nonetheless, our results did not reveal such findings. Specifically, the two perturbation measures tested here did not show an increase associated with estrogen levels. This could suggest that the increased mass of the vocal folds, associated with the raise in estrogens levels during IVF treatment, does not follow a typical pattern of edema or extracellular fluid accumulation. Instead, it is possible that fluids are accumulated intracellularly, such that normal vibratory pattern is not disturbed and voice quality remains unaffected. Clearly, because this hypothesis is based on preliminary results derived from acoustic analyses, it should be further examined and confirmed histologically or by using an appropriate imaging technique.

The second major finding of our study is the negative correlations between estrogen levels and F_0 measures at session I. By itself, this result provides added support to the known effect of increased estrogen levels on lowering F_0 .^{1,4,7} However, this correlation was found only for the natural hormonal climate condition (ie, session I) but not during the other two session. As demonstrated in the Results section, there is a large difference in estrogen levels between the preliminary natural condition and the later conditions. Estrogen levels during a natural cycle reach a value of approximately 940 pmol/L.¹³ This value is substantially lower than the average estrogen level found in our cohort at session III (7464.3 pmol/L). We, therefore, suggest the possibility of a "ceiling effect" for estrogen levels on the vocal folds. Such a ceiling effect was previously reported for other target organs.²⁷ Accordingly, the vocal folds, like other organs, have a maximal thickness capacity, which cannot be transcended. This enforces a limit on the maximal impact of estrogen levels on acoustic properties of the female voice.

The negative correlation between F_0 measures and endometrial thickness at session III suggests that increased estrogen levels affect both endometrium and vocal folds similarly. This finding lends support to previous well-documented reports on cytological similarity between vocal fold's mucosa and vaginal and endometrial mucosa.^{1,2,5} This correlation, however, was only found close to the end of the ovarian stimulation phase (ie, session III). It could, therefore, be interpreted as suggesting a difference in the time required for reaching a clinical effect of these estrogen levels on the endometrium, in comparison to its parallel effect on the vocal folds.

Dividing the participants to two subgroups, based on final endometrial thickness, revealed consistent and significant voice differences, even before the IVF treatment. These differences were statistically significant, despite the relatively small number of women in each subgroup. This suggests that as women's voices convey acoustic indications related to their vocal folds' mucosa characteristics,^{2,24} it can also provide indications associated with endometrium characteristics. Final endometrial thickness after IVF treatment was previously shown to be predictive of final treatment outcome.²⁸ Thus, it is possible that specific acoustic voice measures obtained at baseline could serve as a supplementary indication of the likelihood for final treatment outcome. To test this possibility, future research should examine it directly, on a larger sample, considering additional factors that could affect endometrial thickness and, with a wider set of measures, that would provide elaborated representation of voice characteristics and dynamics.

Finally, it should be clarified that all acoustic measurements obtained in this study were within expected normal range.²⁹ First, this provides added validation to the performed measurements and to the study protocol. Second, this could resolve the seeming disagreement between the conclusions drawn from the previous study using a perceptual paradigm¹⁶ and those drawn from our study. Specifically, because the acoustic values obtained during the three recording sessions were within normal range, they are all expected to be perceived by listeners as normal. Furthermore, our participants did not report of any voice problems during the IVF treatment, and their VHI-10 scores were typical of nondysphonic speakers. Therefore,

listeners, as well as the speakers themselves, are not likely to identify vocal changes throughout the treatment or between subgroups. Hence, we suggest that identification of these hormonal-induced effects should not be performed perceptually. Instead, an acoustic analysis approach should be used for this purpose in the future because it provides a more accurate, sensitive, and reliable paradigm within this context.

CONCLUSION

In this preliminary study, specific vocal changes were documented acoustically in women undergoing IVF treatment. In addition, a ceiling effect for the influence of estrogens on female vocal folds was evident.

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