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Does *in vitro* fertilization affect the hearing levels of women?

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Abstract. – OBJECTIVE: This study aimed to determine whether there is a relationship between changes in blood estrogen levels and hearing threshold levels in women undergoing *in vitro* fertilization (IVF).

PATIENTS AND METHODS: Sixty patients with normal otoscopic examination findings and scheduled for IVF treatment were included in the study. All patients underwent pure tone audiometry, tympanogram tests, and otoacoustic emission measurements (TEOAE) during IVF treatment, and estrogen levels were measured simultaneously. The patients were divided into three groups based on estrogen levels during IVF. The patients' audiological test results at the beginning and end of treatment (the 12th day) were compared between the groups.

RESULTS: There was an increase in hearing levels on the 12th day of treatment compared to the beginning of treatment in all the study groups. This increase was significant between Group 1 and Group 2 and between Group 1 and Group 3 ($p<0.05$). The best hearing in audiometry was determined in the patients in Group 2.

While a significant increase was observed in TEOAE (otoacoustic emission) measurements in Groups 1 and 2, a significant decrease was determined in Group 3 ($p<0.05$). Middle ear pressure and compliance measurements on the tympanogram tests were significantly lower in Group 3 compared to Groups 1 and 2 ($p<0.05$).

CONCLUSIONS: During IVF treatment, as the estrogen level increases, it causes an increase in the level of hearing, but this increase does not continue after specific doses.

Key Words:
Audiogram, Estrogen, *In vitro* fertilization, TEOAE, Tympanogram.

Introduction

Estrogen is a female sex hormone: the blood levels vary in a circadian rhythm during the menstrual cycle. It may exhibit a stimulating and neuroprotective effect on auditory nerve fibers[1,2]. Alpha and beta estrogen receptors have been identified in spiral ganglion type I cells, the stria vascularis, and cochlear blood vessels in the inner ear. Estrogen can thus affect auditory conduction by modulating the fluid-electrolyte balance in cochlear fluids and cochlear blood flow[3]. It also exhibits an antioxidant effect as a free radical scavenger[4].

In vitro fertilization (IVF) is a form of assisted reproductive technology designed to overcome fertility and achieve a live birth. It generally involves stimulating the ovaries with a combination of fertility drugs, retrieving oocytes from ovarian follicles, and fertilizing them in the laboratory *in vitro*[5]. Initial estrogen levels of 50-80 pg/ml can rise to 2,000-4,000 pg/ml during IVF treatment[6].

Several human and animal studies in literature have investigated the effects of estrogen on hearing levels. However, no studies have examined the audiological outcomes of rising estrogen levels in IVF patients.

This study aimed to determine whether changes in blood estrogen associated with IVF affect hearing threshold levels.

Patients and Methods

The Ethical Committee of the University of Health Sciences Erzurum City Hospital, Erzurum, Turkey, approved the study. The informed consent form was obtained from all patients. The research was conducted at the Atatürk University Medical Faculty Ear Nose and Throat (ENT) and Gynecology and Obstetrics departments.

Patient Selection and Groups

The study commenced with 65 patients scheduled for IVF treatment. However, five patients were excluded, three due to ovarian hyperstimulation syndrome and two due to inability to obtain

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oocytes. The study was thus completed with 60 patients. Antagonistic therapeutic protocols were applied using r-FSH (Gonal-F, Serono, Genova, Switzerland) and/or hp-hMG (Menopur, Ferring, Saint-Prex, Sweden) for the induction of ovulation in IVF treatment. A flexible protocol was applied in the antagonist protocol, with 0.25 mg/day cetrorelix (Cetrotide, Merck-Serono, Darmstadt, Germany) and ganirelix (Fresenius, Zurich, Switzerland) employed as antagonists. Before induction, endometrium thickness was measured on days 2-3 of the menstrual cycle using transvaginal ultrasonography (TVUSG), and antral follicle numbers were determined. The initial gonadotropin dosage was decided based on the patient's ovarian reserves, basal hormone status, and antral follicle numbers. The initial gonadotropin dose was 150 or 225 IU. Follicle sizes and numbers were determined using TVUSG, and serum estradiol levels were measured at 1-3-day intervals from days 3-5 from induction. Human chorionic gonadotropin (hCG) was administered on days 11 or 12 to trigger ovulation when the leading follicle reached 18 mm in size or when two strands 17 mm or longer were observed. Urinary hCG (Pregnyl, Organon, Jersey City, USA) 5,000/10,000 IU or 250 µg recombinant hCG (Ovitrelle, Serono, Italy) was used to trigger ovulation. The OPU procedure was performed 34-36 hours after ovulation had been started. Embryos obtained using IVF were transferred on the third day. Intravaginal progesterone (Crinone 8% gel, Serono, Zurich, Switzerland) once daily or intravaginal micronized progesterone 3×200 mg (Progestan soft capsule, Koçak, Istanbul, Türkiye) were applied 24-48 h after OPU and were maintained for 12 weeks in the event of pregnancy being achieved. Pregnancy was confirmed by measuring β hCG values 14 days after embryo transfer. Serum β hCG positivity was regarded as a chemical pregnancy, observation of an intrauterine gestational sac with fetal heartbeat at ultrasonography 5-6 weeks after transfer as clinical pregnancy, and at least one live fetus at the end of 10-11 weeks following transfer as continuing pregnancy. Right and left ear tests were performed separately on all patients with natural otoscopic examinations in the ENT clinic. Estrogen levels were investigated simultaneously on days 2 and 12 with the patients' hearing evaluations. All hearing tests were performed at the same time of day in all subjects to control the effects of physiological circadian variations. Pure tone audiometry, tympanogram tests, and otoacoustic emission measurements (TEOAE) were performed at the beginning and end of IVF treatment. Right and left ear measurements were performed by applying 500, 1,000, 2,000, and 4,000 Hz frequency stimuli on the audiogram test. The patients were aged 20-40 years and were divided into three groups based on estrogen levels measured on the 12th day - Group 1: 0-999 pg/m (17 patients); Group 2: 1,000-2,999 pg/ml (26 patients); Group 3: 3,000 pg/ml or above (17 patients).

Exclusion Criteria

Patients with non-normal otoscopic examinations, with previous hearing level problems, with outer ear canals blocked by cerumen, with serous otitis, receiving medical treatment due to ear infections, with additional diseases, with genital infections, ovarian insufficiency, Turner syndrome, hypothyroidism, or Stein-Leventhal syndrome, menopausal women, pregnant women, and lactating mothers, women in early adolescence, using steroid hormones, and with histories of treatment for depression, head trauma, stroke, heart attack, endocrine function disorders, metabolic, neoplastic pathologies, anorexia nervosa, liver cell necrosis, harmful levels of nicotine or alcohol use, or alcoholic liver disease were excluded from the study. This information was retrieved from the medical records and case histories.

Chemical Substances, Systems, and Devices Employed

Following collection, blood specimens were centrifuged at 4,000 rpm for 10 min, and the resulting sera were separated. Estrogen levels were investigated on a Beckman Coulter DXI800 device (Beckman Coulter Inc., Brea, CA, USA) using the chemiluminescence method. Hearing threshold levels were determined using the pure tone average at a frequency range of 500-4,000 Hz with a pure tone audiometry Maico MA 53 model device (Maico, Berlin, Germany). Tympanogram compliance and pressure measurements were performed using an Interacoustics AT 2354 device and a 300±600 data range. Instantaneous evoked otoacoustic emission (TEOAE) measurements were conducted with a Vivasonic VivoLink model Bera device (Vivasonic, Toronto, Canada) at 1, 2, 3, and 4 kHz frequencies.

Statistical Analysis

Statistical analyses were performed on SPSS software, version 20 (IBM Corp., Armonk, NY, USA). Data were expressed as mean, standard deviation, median, minimum, maximum, percentage, and number. The normality of the

distribution of continuous variables was evaluated using the Shapiro-Wilk test, the Kolmogorov-Smirnov test, Q-Q plots, skewness, and kurtosis. Comparisons between two independent groups were performed using the Independent Samples *t*-test in case of normal distribution and the Mann-Whitney U test in case of non-normal allotment. Comparisons of continuous variables between more than two independent groups were performed using the ANOVA test in case of normal distribution or the Kruskal-Wallis' test in case of non-normal allotment. Post-hoc tests after the ANOVA test were performed using Tukey's test when variances were homogeneous and with the Tamhane T2 test when clashes were non-homogeneous. The Kruskal-Wallis one-way ANOVA (k samples) test was used for post-hoc tests following the Kruskal-Wallis test. The repeated measures ANOVA test was applied to compare more than two independent group variables when the normality of distribution was established, and the Friedman test was used in the case of non-normal allotment. The sphericity assumed, or Greenhouse-Geisser methods, were employed depending on repeated measures of test sphericity. Post-hoc tests after the repeated measures test were performed using Tukey's test when variances were homogeneous and Tamhane's T2 test when they were non-homogeneous. The Friedman two-way ANOVA by ranks (k samples) test was applied for post-hoc tests following the Friedman test. *p*-values <0.05 were regarded as statistically significant.

Results

This study was completed with 60 patients who received IVF treatment. These were between 20 and 40, with a mean age of 29.07±5.48. The patients' estrogen levels were min: 3, max: 5,522 pg/ml. The minimum estrogen level on the first day of treatment was 3 pg/ml, and the maximum was 277 pg/ml. The mean estrogen level on the first day was 53.20 pg/ml. The standard deviation was 47.44. The minimum estrogen level on the 12th day of treatment was 405 pg/ml, and the highest level was 5,522 pg/ml. The mean estrogen level on day 12 was 2,367 pg/ml, with a standard deviation of 1,316.96 (Table I).

Pure Tone Audiometry

Audiometric evaluation of the 60 patients who had received IVF treatment and whose estrogen levels had risen revealed an increase in all the groups on the 12th day of treatment compared to baseline. That increase was statistically significant between groups 1 and 2 and between groups 1 and 3 ($p<0.05$), but was not crucial between groups 2 and 3 ($p>0.05$). The best hearing in audiometry was determined in the patients in Group 2. Increases in audiometry were observed in both ears and air and bone pathways in all groups, particularly at frequencies of 2,000 Hz and 4,000 Hz (Figure 1, Figure 2, and Table II, Table III).

Otoacoustic Emissions

Comparison of TEOAE measurements between baseline and at the end of treatment revealed a significant increase at all frequencies and in both ears in groups 1 and 2, particularly at frequencies of 1 kHz and 4 kHz ($p<0.05$). However, a significant decrease was observed at all frequencies and in both ears in the patients from Group 3 ($p<0.05$). The highest increase was observed in Group 2 (Figure 3, Figure 4, Figure 5, and Table IV).

Tympanometry

Comparison of tympanogram test values at the end of treatment and baseline revealed no statistically significant change in compliance measurements in Groups 1 and 2. However, a significant decrease was observed in patients in Group 3 compared to Groups 1 and 2 ($p<0.05$).

A comparison of tympanogram test values at the end of treatment and baseline revealed no significant change in middle ear pressures in patients from Groups 1 or 2. However, pressures decreased significantly in Group 3 compared to groups 1 and 2 ($p<0.05$).

Table I. The mean age and estrogen levels of the patients in the study.

	N	Minimum	Maximum	Mean	Standard deviation
Age	60	20	40	29.07	5.48
Estrogen (1st day of treatment) pg/ml	60	3	277	53.20	47.44
Estrogen (12th day of treatment) pg/ml	60	405	5,522	2,367.17	1,316.96

Figure 1. Pure-tone audiometry of airway analysis results, considering mean increase hearing threshold at each frequency and comparison between before treatment and after treatment for each ear.

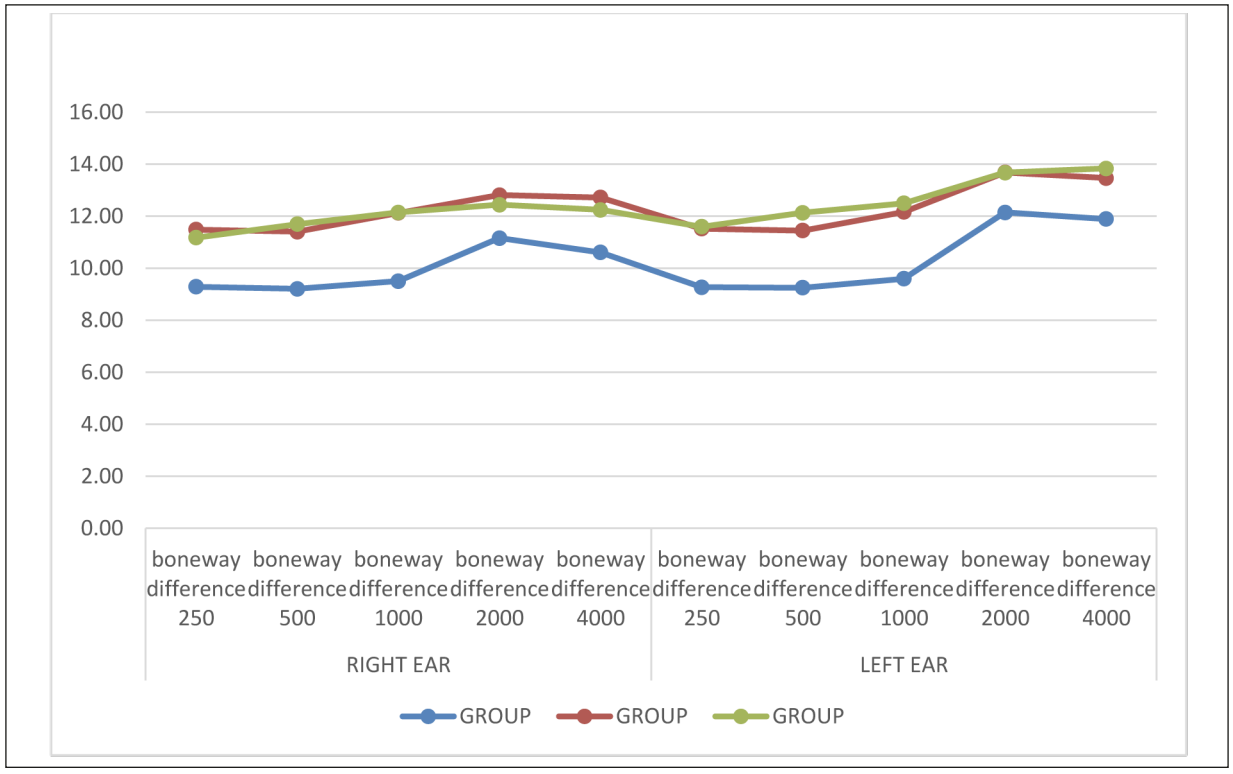

Figure 2. Pure-tone audiometry of boneway analysis results, considering mean increase hearing threshold at each frequency and comparison between before treatment and after treatment for each ear.

Table II. Audiometric result in both ears and in air ways in all groups before and after treatment.

			Right						Left					
			Before		After				Before		After			
			mean±std	median (min-max)	mean±std	median (min-max)	*t*	*p*	mean±std	median (min-max)	mean±std	median (min-max)	*t*	*p*
0-999 pg/ml	Air	250 Hz	15.73 ± 3.1	15.5 (11-22)	6.43 ± 3.01	6.5 (2-12)	70.301	0.000	16.35 ± 2.92	16 (12-23)	7.17 ± 3.1	7 (3-13)	44.609	0.000
		500 Hz	16.35 ± 3.1	16.5 (11.5-21.5)	6.84 ± 3.13	7 (2-11)	59.975	0.000	17.04 ± 2.83	17 (12.5-22)	7.52 ± 3.21	8 (3-12)	41.590	0.000
		1,000 Hz	16.45 ± 3.42	17 (11-22.4)	6.96 ± 3.37	7.25 (2-12.4)	55.553	0.000	17.21 ± 3.14	17.5 (12-23.5)	7.72 ± 3.4	8 (3-13.4)	35.392	0.000
		2,000 Hz	16.42 ± 3.43	16.5 (10-21.8)	5.59 ± 2.64	5.5 (2-10)	42.934	0.000	17.63 ± 3.01	18.7 (11.6-22)	5.44 ± 2.63	5 (2-10)	43.404	0.000
		4,000 Hz	16.36 ± 3.47	17.15 (10-21.7)	5.79 ± 2.91	6.1 (1.5-10)	46.580	0.000	17.57 ± 3.48	18.7 (11-22.8)	5.51 ± 3.04	6 (1-10)	27.797	0.000
1,000-2,999 pg/ml		250 Hz	15.3 ± 2.9	15.75 (10-22)	3.69 ± 2.29	3.75 (1-10)	47.426	0.000	16.3 ± 2.9	16.75 (11-23)	4.69 ± 2.29	4.75 (2-11)	47.426	0.000
		500 Hz	15.66 ± 2.92	16 (10.5-21.5)	3.96 ± 2.48	3.75 (1-11)	48.074	0.000	16.62 ± 2.93	17 (11.5-22.5)	4.98 ± 2.5	4.75 (2-12)	48.954	0.000
		1,000 Hz	16.08 ± 2.59	16.5 (10.8-22.4)	3.98 ± 2.41	3.5 (1-12)	50.239	0.000	17.03 ± 2.57	17.5 (11.8-23.4)	4.98 ± 2.41	4.5 (2-13)	48.463	0.000
		2,000 Hz	16.53 ± 2.77	17 (10-22.8)	3.44 ± 2.18	3 (1-10)	49.510	0.000	17.56 ± 2.77	18 (11-23.8)	3.48 ± 2.21	3 (1-11)	44.338	0.000
		4,000 Hz	16.36 ± 2.82	16 (10-23.7)	3.59 ± 2.27	3 (1-10)	43.874	0.000	17.02 ± 2.67	17 (11-24.7)	3.55 ± 2.16	3 (1-10)	27.555	0.000
3,000 and sbove		250 Hz	15.88 ± 2.49	17 (10.5-19)	4.14 ± 2.11	5 (1-7)	72.847	0.000	16.91 ± 2.42	18 (12-20)	5.11 ± 2.08	6 (2-8)	82.720	0.000
		500 Hz	15.85 ± 2.6	16 (8-19)	4.08 ± 1.71	4 (1-6.5)	35.246	0.000	16.87 ± 2.61	17 (9-20)	5.15 ± 1.77	5 (2-8)	35.151	0.000
		1,000 Hz	15.94 ± 2.87	16.5 (8-19)	4.21 ± 1.85	4.5 (1-7)	29.668	0.000	16.94 ± 2.87	17.5 (9-20)	5.27 ± 1.87	5.5 (2-8)	29.554	0.000
		2,000 Hz	16.44 ± 3.03	17 (9.5-22)	3.76 ± 2.07	4 (1-9)	36.835	0.000	17.51 ± 3.01	18 (10.5-23)	3.95 ± 2.01	4 (1-9)	33.681	0.000
		4,000 Hz	15.94 ± 3.28	17 (9-23)	3.94 ± 2.06	4 (1-9)	32.536	0.000	16.94 ± 3.28	18 (10-24)	4.12 ± 2.04	4 (1-9)	34.500	0.000

Table III. Audiometric result in both ears and in bone ways in all groups before and after treatment.

		Right						Left					
		Before		After				Before		After			
		mean±std	median (min-max)	mean±std	median (min-max)	*t*	*p*	mean±std	median (min-max)	mean±std	median (min-max)	*t*	*p*
0-999 pg/ml	Bone 250 Hz	14.63 ± 2.94	14.55 (10-19)	5.34 ± 2.8	5.5 (1-10)	88.595	0.000	15.35 ± 3.06	15.1 (11-20)	6.08 ± 2.91	6 (2-11)	92.786	0.000
	500 Hz	14.95 ± 3.01	15.25 (10-19.8)	5.75 ± 3.04	6 (1-10)	55.061	0.000	15.72 ± 3.07	15.5 (11-20.8)	6.47 ± 3.16	7 (2-11)	56.472	0.000
	1,000 Hz	15.25 ± 3.19	15.25 (11-21)	5.75 ± 3.25	6 (1-11)	53.388	0.000	16.06 ± 3.19	16 (12-22)	6.47 ± 3.35	7 (2-12)	50.780	0.000
	2,000 Hz	15.34 ± 3.5	15.75 (9-21)	4.18 ± 2.58	4 (1-9)	36.699	0.000	16.08 ± 3.28	16.5 (10-21)	3.94 ± 2.53	4 (1-9)	42.516	0.000
	4,000 Hz	15.04 ± 3.35	15.7 (9.5-19.8)	4.43 ± 2.8	4 (1-9)	34.359	0.000	15.95 ± 3.2	16 (10.5-20.5)	4.05 ± 2.6	4 (1-9)	31.902	0.000
1,000-2,999 pg/ml	250 Hz	14.25 ± 2.65	15 (9-19)	2.76 ± 2.04	2 (1-9)	37.442	0.000	15.25 ± 2.65	16 (10-20)	3.73 ± 1.92	3 (2-9)	38.200	0.000
	500 Hz	14.19 ± 2.76	14.25 (9-19.8)	2.78 ± 2.08	2 (1-9)	41.954	0.000	15.19 ± 2.76	15.25 (10-20.8)	3.75 ± 1.97	3 (2-9)	42.207	0.000
	1,000 Hz	14.74 ± 2.53	15 (9.5-21)	2.61 ± 1.85	2 (1-8)	40.447	0.000	15.74 ± 2.53	16 (10.5-22)	3.57 ± 1.74	3 (2-8)	39.667	0.000
	2,000 Hz	15.12 ± 2.72	15.25 (9-21)	2.3 ± 1.71	2 (1-8)	34.592	0.000	16.12 ± 2.72	16.25 (10-22)	2.44 ± 1.76	2 (1-8)	36.207	0.000
	4,000 Hz	14.89 ± 2.63	15 (9.5-21)	2.17 ± 1.58	2 (1-7)	37.824	0.000	15.87 ± 2.56	16 (11-22)	2.4 ± 1.53	2 (1-7)	43.550	0.000
3,000 and above	250 Hz	14.05 ± 2.44	14 (8-17)	2.88 ± 1.61	3 (1-6)	27.186	0.000	15.47 ± 2.64	16 (9-19)	3.88 ± 1.61	4 (2-7)	25.325	0.000
	500 Hz	14.34 ± 2.56	15 (7-17)	2.64 ± 1.61	3 (1-6)	26.405	0.000	15.78 ± 2.72	16.5 (8-19)	3.64 ± 1.61	4 (2-7)	23.857	0.000
	1,000 Hz	14.67 ± 3.02	15.5 (6-18)	2.52 ± 1.66	2 (1-6)	20.957	0.000	16.02 ± 3.11	17 (7-19)	3.52 ± 1.66	3 (2-7)	19.701	0.000
	2,000 Hz	14.73 ± 3	16 (8-19)	2.29 ± 1.31	2 (1-5)	22.949	0.000	16.08 ± 3.1	17 (9-20)	2.41 ± 1.22	2 (1-5)	21.657	0.000
	4,000 Hz	14.6 ± 3.51	15 (8-22)	2.35 ± 1.16	2 (1-5)	18.868	0.000	16.18 ± 3.83	18 (9-24)	2.35 ± 1.11	2 (1-5)	18.166	0.000

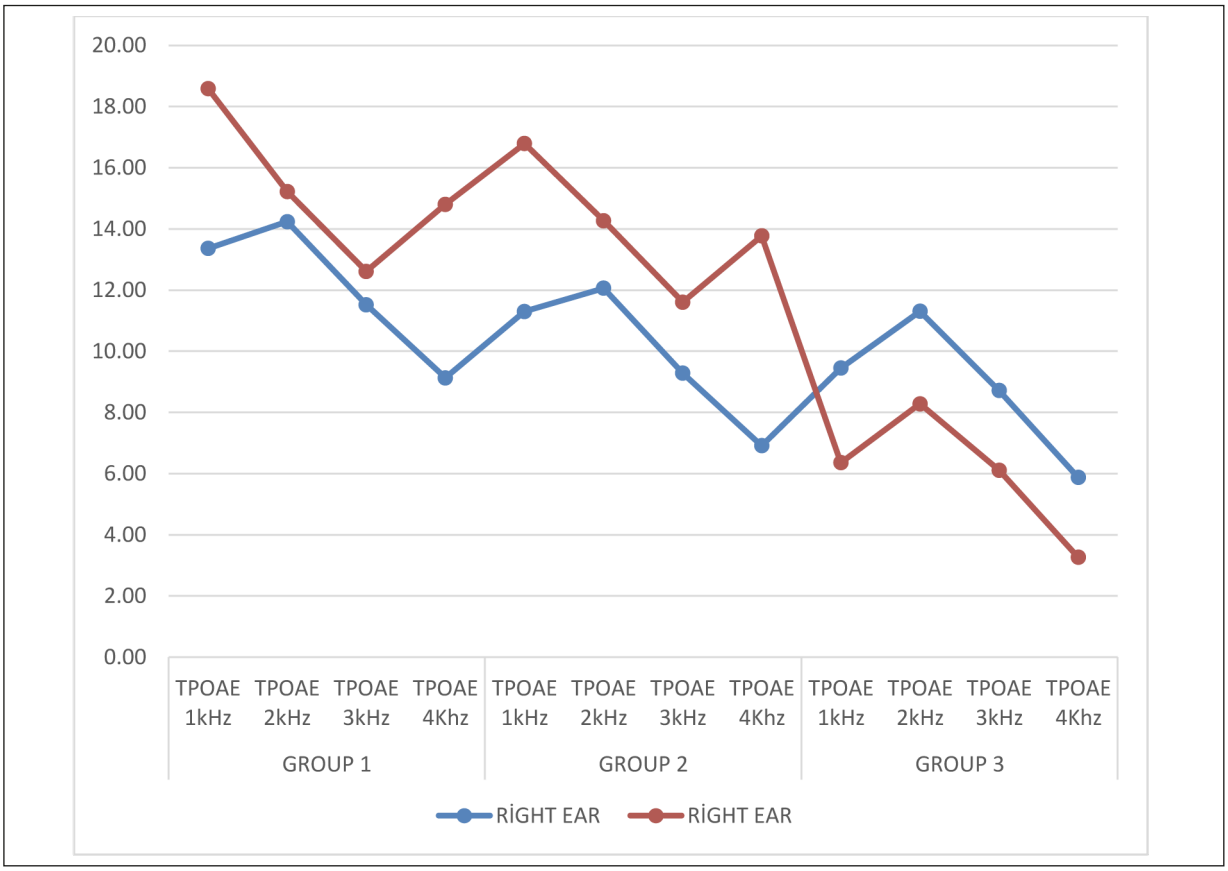

Figure 3. TPOAE analysis, considering mean amplitudes at each frequency and comparison between treatment before and after for right ear.

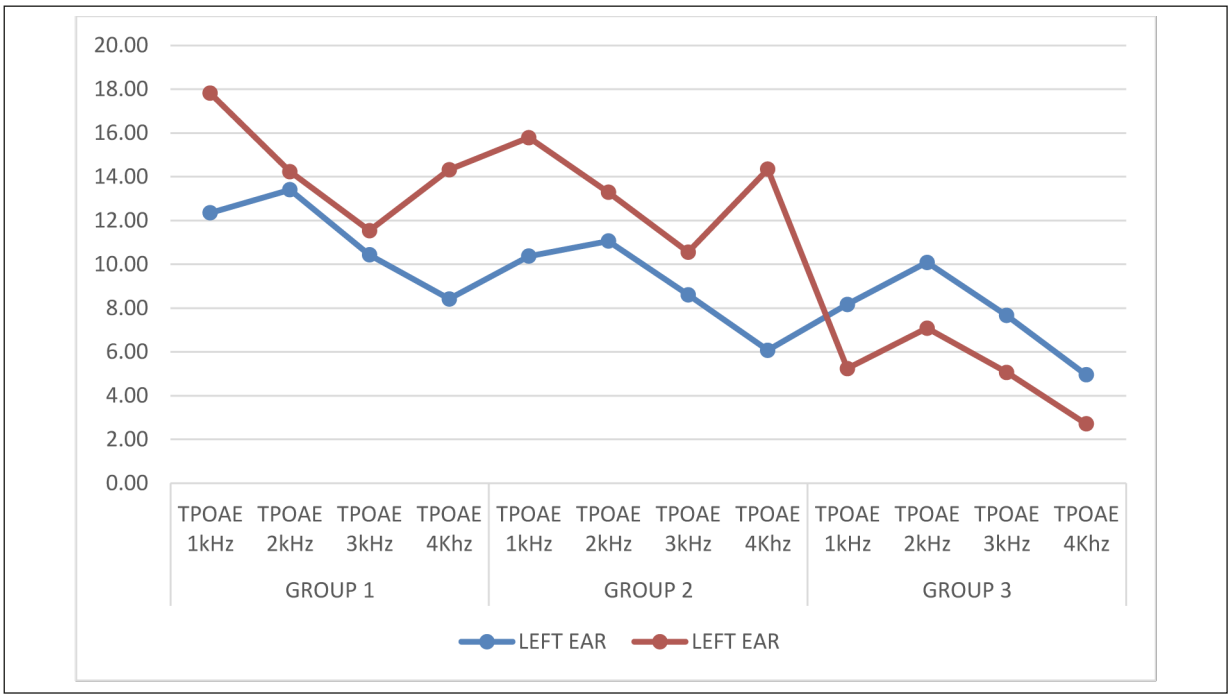

Figure 4. TPOAE analysis, considering mean amplitudes at each frequency and comparison between treatment before and after for left ear.

	RIGHT EAR				LEFT EAR			
	difference TPOAE 1kHz	difference TPOAE 2kHz	difference TPOAE 3kHz	difference TPOAE 4kHz	difference TPOAE 1kHz	difference TPOAE 2kHz	difference TPOAE 3kHz	differenceTPOAE 4kHz
GROUP	5.23	0.99	1.09	5.68	5.46	0.83	1.11	5.91
GROUP	5.49	2.21	2.33	6.86	5.42	2.22	1.95	8.27
GROUP	-3.09	-3.02	-2.61	-2.61	-2.93	-3.01	-2.61	-2.25

Figure 5. The difference in gain in TPOE values in the right and left ears before and after treatment between the groups.

Discussion

According to our findings, while the rise in blood estrogen levels is correlated with improvement in hearing threshold levels up to specific doses, this correlation does not persist at higher doses. In other words, the increased blood estrogen levels in IVF patients improve hearing but do not exhibit the same effect at higher doses.

Although the relationship between the hormone estrogen and hearing function has been extensively examined in humans and animals, the effects of estrogen on hearing levels remain unclear.

The literature[7] concerning the mechanism by which sex hormones alter auditory thresholds points to two probable effect types: (1) direct modulation through various pathways in the cochlea and central auditory system and (2) modulation of blood flow and hemostasis in the cochlea and brain.

The availability of various neurotransmitters (such as Ach and GABA) in synapses in the auditory pathways is believed to depend on estrogen levels, suggesting that estrogen also has an essential effect on sound transmission times[8-10].

Homeostasis and the biochemical status of inner ear fluid are essential for balance and hearing. Changes occurring in sodium and water reabsorption due to estrogen and progesterone levels may affect the functioning of the peripheral auditory system by impacting homeostasis, which can give rise to auditory and labyrinth symptoms.

Blood E2 (estrogen) values of 15-350 pg/mL are regarded as usual for women of reproductive age; they can range between 40 and 2,000 pg/mL[11-13]. During pregnancy, estrogen is released slightly higher during the first three months than between ovulation and menstruation. After the first three months, levels rise to 30-50 times higher than usual. The E2 levels of women who received IVF treatment in the present study increased in as little as 12 days, and the effect of these high E2 levels on hearing threshold values during that period was investigated[11-13].

Several protocols are widely employed for IVF treatments. In the present study, antagonist protocols using r-FSH (Gonal-F, Serono, Switzerland and Puregon, Organon, Jersey City, USA) and/or hp-hMG (Menopur, Ferring, Sweden) were applied for ovulation induction.

Numerous studies[11-13] have investigated the effects on the auditory system of physiological fluctuations in reproductive hormones, including

Table IV. TPOAE. Compliance and pressure result in both ears and in all groups before and after treatment.

		Right						Left					
		Before		After				Before		After			
		mean±std	median (min-max)	mean±std	median (min-max)	*t*	*p*	mean±std	median (min-max)	mean±std	median (min-max)	*t*	*p*
0-999 pg/ml	TPOAE 1 kHz	13.36 ± 0.37	13.35 (12.8-14)	18.58 ± 0.56	18.5 (17.8-19.4)	-42.138	0.000	12.35 ± 0.36	12.3 (11.8-13)	17.81 ± 0.61	18.1 (16.8-18.6)	-38.295	0.000
	2 kHz	14.23 ± 0.68	14.4 (13.3-15.2)	15.22 ± 0.7	15.25 (14.3-16.2)	-13.328	0.000	13.4 ± 0.68	13.6 (12.3-14.4)	14.23 ± 0.68	14.5 (13.3-15.2)	-5.825	0.000
	3 kHz	11.52 ± 2.31	11.05 (8.6-16.5)	12.61 ± 2.32	12.1 (9.7-17.6)	-147.486	0.000	10.42 ± 2.27	9.6 (7.6-15.5)	11.53 ± 2.27	10.9 (8.7-16.6)	-87.182	0.000
	4 Khz	9.12 ± 2.92	8.3 (4.9-13.9)	14.8 ± 2.99	14 (10.2-19.4)	-53.598	0.000	8.41 ± 3.09	7.4 (3.9-13.9)	14.32 ± 3.12	13.9 (9.6-18.8)	-43.007	0.000
1,000-2,999 pg/ml	1 kHz	11.3 ± 0.49	11.3 (9.6-12)	16.79 ± 0.57	16.8 (15.8-17.6)	-44.324	0.000	10.36 ± 0.6	10.3 (8.6-12.1)	15.78 ± 0.57	15.8 (14.8-16.5)	-40.237	0.000
	2 kHz	12.06 ± 0.81	11.75 (10.1-13.2)	14.26 ± 0.75	14.2 (13.3-15.7)	-15.807	0.000	11.06 ± 0.81	10.75 (9.1-12.2)	13.28 ± 0.77	13.2 (12.3-14.7)	-15.826	0.000
	3 kHz	9.28 ± 2.58	9 (3.1-14.5)	11.6 ± 2.25	11.1 (8.7-16.6)	-10.414	0.000	8.6 ± 2.26	8.2 (5.5-13.5)	10.55 ± 2.28	9.95 (7.7-15.6)	-9.757	0.000
	4 khz	6.91 ± 2.79	6.4 (2.9-11.9)	13.77 ± 2.81	13.35 (9.6-18.7)	-65.599	0.000	6.07 ± 2.85	5.95 (1.9-10.9)	14.34 ± 2.77	13.8 (10.2-19.1)	-43.970	0.000
3000 and above	1 kHz	9.45 ± 0.56	9.3 (8.8-11.1)	6.35 ± 0.91	6.3 (4.5-8.3)	23.717	0.000	8.16 ± 0.93	8.3 (4.8-9)	5.23 ± 0.76	5.3 (3.5-7)	14.801	0.000
	2 kHz	11.3 ± 0.88	11.5 (10.3-13.4)	8.28 ± 0.84	8.5 (7.3-10.1)	108.663	0.000	10.08 ± 0.79	9.8 (8.7-11.2)	7.08 ± 0.78	6.7 (5.7-8.2)	137.806	0.000
	3 kHz	8.71 ± 2.48	8.6 (5.5-13.5)	6.11 ± 2.46	6.5 (2.5-10.5)	10.520	0.000	7.66 ± 2.5	7.2 (4.5-12.5)	5.05 ± 2.48	5.5 (1.5-9.5)	10.520	0.000
	4 Khz	5.87 ± 3	5.3 (1.9-10.9)	3.26 ± 2.46	2.4 (1-7.5)	13.410	0.000	4.95 ± 2.98	4.4 (1-9.9)	2.7 ± 2.1	1.4 (1-6.5)	7.897	0.000
0-999 pg/ml	Complians	0.54 ± 0.05	0.55 (0.45-0.66)	0.54 ± 0.04	0.53 (0.47-0.61)	1.000	0.333	0.53 ± 0.03	0.53 (0.47-0.61)	0.52 ± 0.03	0.52 (0.46-0.61)	3.250	0.005
1,000-2,999		0.53 ± 0.05	0.54 (0.45-0.66)	0.53 ± 0.04	0.52 (0.45-0.61)	0.984	0.334	0.53 ± 0.04	0.52 (0.45-0.61)	0.52 ± 0.03	0.52 (0.45-0.61)	1.850	0.076
3,000 and above		0.54 ± 0.05	0.55 (0.45-0.66)	0.41 ± 0.01	0.53 (0.47-0.61)	8.918	0.000	0.53 ± 0.04	0.53 (0.47-0.61)	0.42 ± 0.02	0.43 (0.35-0.45)	10.923	0.000
0-999 pg/ml	Pressure	7.93 ± 8.16	9 (-10-20)	5.56 ± 8.6	6 (-12-18)	5.836	0.000	8.35 ± 5.94	9 (-8-16)	5.94 ± 8.15	8 (-10-16)	1.185	0.253
1,000-2,999 pg/ml		8.34 ± 6.32	10 (-8-16)	5.92 ± 8.47	9 (-10-16)	1.327	0.196	8.34 ± 6.32	10 (-8-16)	5.92 ± 8.47	9 (-10-16)	1.327	0.196
3,000 and above		7.93 ± 8.16	9 (-10-20)	-64.5 ± 11.47	-61.5 (-82 – -50)	23.229	0.000	6.87 ± 7.03	9 (-8-16)	-73.87 ± 9.39	-76 (-86 – -58)	42.833	0.000

in patients undergoing hormone replacement therapy, menopausal women, women using hormonal contraception, and during the ovarian cycle.

Indri et al[14] observed no association between the menstrual cycle and audiometric hearing thresholds but reported a relationship between the menstrual cycle and outer hair cell function[14].

However, Yellin and Stillman[15] reported that short-term hormone variations in the menstrual cycle produce changes in a woman's body. However, these brief variations do not cause significant alterations in transient otoacoustic emissions or distortion responses.

A study[16] including 20 healthy women with regular menstrual cycles (age 19±30 years) investigated hearing on the 13th day of the menstrual cycle (the follicular phase) and then on the 22nd day (the luteal stage). The authors measured estradiol levels > 600 pg/ml in the follicular phase and < 250 pg/ml in the luteal phase and reported average hearing results in the follicular phase in all participants. They concluded that changes in ovarian hormones may only lead to abnormal auditory test results in some susceptible women and that this is due to individual differences[16]. Moreover, Swanson and Dengerink[17] reported better hearing thresholds only at 4,000 Hz during ovulation[17].

Some studies[18-20] have also suggested that decreased estrogen is associated with reduced auditory pathway performance and an increased incidence of neurodegenerative disorders.

Studies on women with congenitally low estrogen levels and postmenopausal individuals have reported delays in auditory brainstem response (ABR) waves and higher thresholds[18,19]. Another study reported delayed ABR responses in ovariectomized animals[7]. An analysis of mice with natural estrogen deficiency showed longer ABR latencies than control mice[20]. These studies suggested that internal estrogen levels may affect hearing thresholds and delayed ABR peaks.

Estrogen has also been shown[21,22] to protect against exposure to noise and glutamate toxicity, to provide protection against oxidative stress by increasing the production of the potent antioxidants superoxide dismutase 2 and hydrogen sulfide, and to reduce the formation of reactive oxygen species[23,24].

Studies[25-29] involving postmenopausal women have shown that menopausal hormonal treatment (MHT) is beneficial against hearing loss but that no benefit in terms of hearing was observed from estrogen therapy alone in patients with Turner syndrome and postmenopausal individuals[30-32].

In addition, estrogen has been reported[3,33] to play a more critical role in protecting normal hearing in young and middle-aged women compared to postmenopausal individuals. It has also been suggested[3,33] that high estrogen levels during the follicular phase in young adult women are likely responsible for better neural conduction and faster conduction times. The mean age of the patients in the present study was 29.7 years.

Wronski et al[34] applied estrogen therapy to ovariectomized rats. They reported that the lowest dose was sufficient and that higher doses of estrogen did not improve the therapeutic effect.

Kim et al[35] investigated the probability of dose-dependent effects of estrogen replacement therapy. They compared hearing thresholds and histological changes in groups of ovariectomized rats exposed to noise receiving ten ug/kg and 100 ug/kg doses of estrogen. After two weeks, both ABR and histological data showed that estrogen replacement therapy improved hearing and increased the density of hair cells in the cochlear independently of the estrogen concentration. Functional and histological analyses revealed no statistically significant difference between the 10 ug/kg and 100 ug/kg groups. The authors suggested that this might be attributable to the low estrogen dose (10 μg/kg) employed in their study already being able to exhibit the maximum possible effect. These results were consistent with those of Wronski et al[34].

However, some animal studies[7,36,37] have reported that exogenously administered estrogen can produce variations in hearing results. This may be associated with the hormone administration regimen, the dose, and the age of the recipients.

In the present study, patients were assigned to Group 1, 0-999 pg/m (17 patients), Group 2, 1,000-2,999 pg/ml (26 patients), and Group 3, 3,000 pg/ml or above (17 patients).

On the 12th day of IVF treatment, the best hearing at audiometry was observed in the patients in Group 2. The audiometric evaluation revealed an increase in all three groups compared to the beginning of treatment. These increases were statistically significant between groups 1 and 2 and between groups 1 and 3 ($p<0.05$), while the difference between groups 2 and 3 was not significant ($p>0.05$).

In light of these results, we concluded that estrogen improves hearing but that higher estrogen levels do not further increase that therapeutic effect and that blood estrogen levels do not exhibit dose-dependent effects in audiometry. These results are similar to those of Kim et al[25].

Tympanometry used in audiological evaluation is an objective test method that yields data concerning the movements of the eardrum and middle ear ossicles, middle ear pressure, and the Eustachian tube through the application of ear pressure to the outer ear canal. The type of tympanogram obtained in normal healthy ears involves a middle ear pressure peaking at between –50 and +50 deka Pascals (data) and eardrum mobility (compliance) within normal limits (mean 0.6 ml)[38].

Comparative analysis of the compliance values of all the patients undergoing tympanogram tests in the present study revealed no change in the patients in Groups 1 or 2 on the 12th day. At the same time, statistically significant decreases were determined in both ears in Group 3. This decrease might be attributable to the congestion and vasodilation in the middle ear and Eustachian tube and increased interstitial fluids caused by high estrogen. Patients in Group 3 described a feeling of fullness in the ears, but no vertigo was present. Previous studies[3,33,39] have also reported that the increase in interstitial fluids in the middle ear and Eustachian tube associated with estrogen may affect the functioning of the Eustachian tube and lead to poorer hearing thresholds.

Otoacoustic emissions (OAEs) can provide robust evidence concerning normal cochlear outer hair cell function. The inner and outer ear must also be normal for detecting OAEs. One study[40] investigating the effects of middle ear pathologies and associated hearing losses on OAEs concluded that abnormal pressures and pathologies in the middle ear reduced the OAE response and increased when appropriate treatment was administered. In general terms, higher OAE means more significant cochlear gain and is associated with better hearing sensitivity.

Yellin and Stillman[15], and Arruda and Silva[41] reported no change in OAE values throughout the menstrual cycles of healthy, non-pregnant women[15,41].

However, Indri et al[14] reported higher DPOAE amplitudes during ovulation in women with regular ovarian cycles than during the follicular and luteal periods. They suggested that this might be attributable to a higher estrogen level during evolution and its positive effect on auditory function[14].

Statistically significant increases in OAE values were detected in both ears and at all frequencies in patients in Groups 1 and 2 on the 12th day of the present study ($p<0.01$). In contrast, a significant decrease was observed in OAE values at all frequencies and in both ears in the patients in Group 3 compared to the other two groups ($p<0.01$). This diminution in OAE values in Group 3 may result from dysfunction occurring in the middle ear and Eustachian tube due to decreasing compliance and middle ear pressure.

Conclusions

The present research is the first study to examine the effect of high doses of estrogen on hearing threshold levels in humans. It should also be remembered that while some women may experience increased hearing during IVF treatment, fullness in the ear and hearing loss may develop in others. Further studies with more significant patient numbers will now be helpful.

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Conflict of Interest
The authors declare that there is no conflict of interest.

Availability of Data and Materials
Data may be provided on reasonable request to the corresponding author.

Informed Consent
The authors declare that the patients included in the study signed informed consent forms to use their medical information in the studies.

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Authors' Contributions
Nurcan Yoruk: Designed the research and wrote the paper; Berrin Aydın: Designed and performed the study and acquisition of data. Both authors read and approved the final manuscript.

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