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ORIGINAL ARTICLE

Effects of Infertility on Voice in male patients

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Abstract

Purpose In this study, we aimed to determine the differences in normospermic, oligospermic and azoospermic infertile men by performing voice analysis and to discuss this in the light of the literature.

Methods 71 male patients who applied to the urology clinic due to infertility and were then referred to us were included in the study. Hormone analysis and spermiogram were requested from the patients for routine infertility tests. Testosterone, Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin levels of the patients were recorded. Age and spermiogram results were recorded. According to the spermiogram results, the patients were categorized as Group 1 (azoospermic), Group 2 (oligospermic), Group 3 (normospermic). Voice Handicap Index-10 Turkish version (VHI-10) was applied to the patients and the results were recorded.

Results The age of the infertile patients ranged from 20 to 37. The mean age was 28.23. The distribution of the patients was 21 patients in Group 1, 40 patients in Group 2, and 10 patients in Group 3. The mean Testosterone level of the patients was 2.78; mean FSH level 12.14; mean LH level 7.26; mean Prolactin level was 8.1. The mean VHI-10 scores of the patients were 10.52. The fundamental frequency F0 Hz (mean pitch) values of the patients were 176,468; jitter % (frequency perturbation jitter) values average 0.25; shimmer % (amplitude perturbation shimmer) values average 2,322; HNR dB values averaged 24,862.

Conclusions Testosterone is more effective on the voice, especially in male individuals.It would be more logical to think that many hormones, growth factors and local factors are effective instead of a single hormone.

Keywords Male · Infertility · Voice · Voice Quality · Phonation · Hormones

Introduction

Infertility is the inability to conceive for more than 12 months despite unprotected intercourse. Male infertility is the cause in 30% of infertility in couples. The most common cause of male infertility is hypogonadism with a 10% probability, namely low testosterone, high luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels.

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Endogenous hypogonadism is the cause of impaired spermatogenesis, and patients with hypogonadism may present with oligozoospermia or azoospermia. The pulsatile production of gonadotropin-releasing hormone (GnRH) by the hypothalamus stimulates the pituitary and induces the synthesis of LH and FSH from the pituitary. LH and FSH ensure testosterone secretion from the last organ (testicle) and maintain spermatogenesis. There is a negative feedback mechanism here. Moreover, excessive serum prolactin level blocks the effect of LH on Leydig cells and causes low testosterone levels, hypogonadism, and infertility.A spermiogram is an essential test for determining the cause of infertility, in which the sperm parameters; sperm volume, viscosity, morphology, movement, and number can be evaluated. Infertile patients can be subdivided according to the total sperm count as azoospermia (no sperm), oligospermia (< 39 million) and normospermia (> 39 million) [1]. In men, the larynx is also among the affected organs, particularly because testosterone increases the volume and size of the muscles and cartilage at puberty. The most important growth changes are elongation and increase in mass of the thyroarytenoid muscle. Vocal cords lengthen, increase in volume and pitch value decreases. Thus, the male voice begins to settle. With the enlargement of the thyroid cartilage, Adam's apple appears [2]. Today, the examination of patients with voice problems is performed through objective methods such as videostroboscopy, aerodynamic and acoustic analysis, and perceptual evaluation by the clinician, as well as subjective assessments made by the patients themselves. Many measurement methods are present for this purpose, and the most common one is the VHI (Voice Handicap Index) method developed by Jacobson et al. There is also a version of this test adapted to Turkish culture and society, and the internal reliability of the test is significant [3, 4].

In this study, we aimed to determine the differences by performing voice analysis in normospermic, oligospermic, and azoospermic infertile men and to discuss this in the light of the literature.

Materials and methods

71 male patients who applied to XXXX Hospital : University of Health Sciences Adana City Training and Research Hospital due to infertility between 01.07.2021 - 01.09.2021 were included in the study. Hormone analysis and spermiogram were requested from the routine infertility tests of the Urology clinic. Testosterone (reference values 1.75–7.81 ng/mL), Follicle stimulating hormone (FSH, reference values 1.3–19.3 mIU/mL), Luteinizing hormone (LH, reference values 1–9 IU/L), Prolactin (reference values 2.64–13.13 ng/ml) levels were recorded. Age and spermiogram results were recorded. Based on the World Health Organization 2010 classification, the absence of sperm in the ejaculate was accepted as azoospermic, the presence of < 39 million sperm in the ejaculate or the presence of 15 million sperm per milliliter as oligospermic (in our study, the total number of sperm in the ejaculate was taken into account), 39 million or more sperm as normospermic. According to the spermiogram results, the patients were divided into three groups. Azoospermic patients were categorized as Group 1, oligospermic patients as Group 2, and normospermic patients as Group 3. Voice Handicap Index-10 Turkish version (VHI-10) was applied to the patients and the results were recorded. Patients who use cigarettes and other tobacco products, which may impair the quality of the voice, were excluded from the study. Patients with nasal and postnasal drip were excluded from the study. Patients with reflux complaints were excluded from the study. Patients with hormone problems other than testosterone, FSH, LH, and prolactin were excluded from the study.Approval for our study was obtained from XXXX Hospital : University of Health Sciences Adana City Training and Research Hospital Clinical Research Ethics Committee. (Meeting Number: 80, Decision Number: 1403, Date: 06.05.2021)

2.1 Objective sound analysis

Paul Boersma and David Weenink sound analysis system (Praat) is one of the leading sound analysis programs and was used in this study. We used the Praat v.4.1. sound analysis program in our study. With this program, we objectively recorded the fundamental frequency F0 (Hz), jitter (%), shimmer (%), and harmonics-to-noise ratio (HNR dB) values from the physical properties of the sound, free of charge.All participants were seated in a quiet room, and analysis was performed. A high-quality, dynamic, cardioid microphone (Audio Technica at 2020) was placed on a stand 20 cm far from the patients. The analysis results were transmitted to a laptop. Voice samples were elicited by asking each patient to reproduce continuous phonations of the /a/ sound at their usual pitch and loudness levels. The researcher made sure that each participant was comfortable and competent in producing continuous phonations at their usual level. Three continuous phonations (each lasting longer than 3 s) were then recorded. The second output was used for data analysis. To eliminate the effects of onset and offset vocalization, the vocalization of the analysed segment was a 1-second segment in the middle of vowel production. Selected segments were then digitized (50 kHz sampling rate) and analysed using Praat, and we selected four of the Praat acoustic sound parameters. Other Praat parameters were excluded as they were irrelevant to the purposes of the experiment. The fundamental frequency F0 (mean pitch), jitter %, shimmer % and harmonics-to-noise ratio (HNR) were measured on acoustic voice analysis. F0, jitter %, shimmer %, HNR values and VHI-10 were statistically compared between all groups.

2.2 Statistical analysis

In the statistical analysis, descriptive statistics for continuous variables were presented as mean ± standard deviation. The normality of continuous variables was tested with the Shapiro-Wilk and Kolmogorov-Smirnov tests. In the comparison of the two groups, the Mann-Whitney U test was used for the variables that did not fit the normal distribution. For more than two groups, the Kruskal-Wallis test, which is an alternative to the analysis of variance, was used for the variables that did not fit the normal distribution. For pairwise comparisons in post-hoc analysis, the Mann-Whitney U test was used with Bonferroni correction. While examining the linear relationship between continuous variables, the Spearman Correlation coefficient (r-value) was used for variables that did not fit into the normal distribution. The significance level (p-value) for all tests was determined as 0.05. All analyses were made with IBM SPSS 22 package program trial version.

2.3.Theory/Calculation

It is seen that although there are many controversial areas about the effect of hormones on voice, nothing explicit has been put forward. However, our current opinion is that testosterone is more effective on voice, especially in male individuals. There was a very strong positive linear correlation between testosterone and shimmer % ($r = 0.871$, $p < 0.01$). In other words, there was a direct proportional relationship; as one increased, the other increased and decreased as it decreased. There was a moderate negative linear correlation between testosterone and HNR dB ($r = -0.452$, $p < 0.01$). In other words, there was an inverse proportional correlation; as one increased, the other decreased and increased as it decreased.

Table 1 Minimum-maximum values of variables

Variables	Minimum	Maximum	Mean ± Standard deviation
Age	20	37	28,23 ± 6,35
Testosterone	1,92	3,90	2,78 ± 0,56
FSH	10,24	15,10	12,14 ± 1,73
LH	3,54	12,43	7,26 ± 3,12
Prolactine	4	12,90	8,10 ± 3,30
Total Sperm Number	0	134,000,000	26827042,25 ± 38835101,81
VHI-10 Points	0	25	10,52 ± 8,78
Fundamental frequency F0 Hz(Mean Pitch)	103,606	272,714	176,46838 ± 52,76376
Jitter % (frequency perturbation jitter)	0,13	0,54	0,25 ± 0,12
Shimmer % (amplitude perturbation shimmer)	1,252	3,897	2,32201 ± 0,88189
HNR dB	18,605	29,941	24,86255 ± 3,78661

Results

71 male patients were included in the study, and their ages ranged from 20 to 37. The mean age was 28.23. The distribution of the patients was 21 in Group 1, 40 in Group 2, and 10 in Group 3. Total sperm count ranged from 0 to 134,000,000, and the average was 26,827,042.

The testosterone level of the patients ranged from 1.92 to 3.90 with an average of 2.78. The FSH level of the patients ranged from 10.24 to 15.1, and the mean value was 12.14. The LH level of the patients ranged from 3.54 to 12.43, and the mean value was 7.26. The prolactin level of the patients ranged from 4 to 12.9, and the mean value was 8.1.

The VHI-10 scores of the patients ranged from 0 to 25, with a mean of 10.52. The fundamental frequency F0 Hz (mean pitch) values of the patients ranged from 103.606 to 272.714, and the mean value was 176.468. The patients' frequency perturbation jitter values ranged from 0.13 to 0.54, and the mean value was 0.25. The shimmer % (amplitude perturbation shimmer) values of the patients ranged between 1.252 and 3.897, and the mean value was 2.322. The HNR dB values of the patients ranged between 18.605 and 29.941, and the mean value was 24.862. The minimum, maximum and mean values are all shown in Table 1.

Among the three groups, there was a statistically significant difference in terms of FSH ($p = 0.011$), LH ($p = 0.014$), total sperm count ($p < 0.001$), jitter % ($p = 0.003$), and HNR dB ($p = 0.005$) variables.

- For the FSH variable, there was a statistically significant difference between Group 2 and Group 3 ($p = 0.017$). The FSH values were higher in Group 3.
- For the LH variable, there was a statistically significant difference between Group 1 and Group 2 ($p = 0.011$). The LH values were higher in Group 2.

Table 2 Groups and p-values

Variables	Groups			P Values
	Group 1 [Q1:Q3] Median	**Group 2 [Q1:Q3] Median**	**Group 3 [Q1:Q3] Median**	
Age	[23,00:32,00] 24,00	[22,00:37,00] 32,00	[25,00:32,00] 25,00	0,289
Testosterone	[2,55:3,42] 3,10	[2,32:3,10] 2,95	[1,92:3,42] 2,12	0,102
FSH	[10,54:14,70] 13,20	[10,54:11,40] 11,11	[11,40:14,70] 14,70	0,011
LH	[3,67:7,54] 6,48	[4,35:12,43] 7,54	[3,67:8,72] 8,72	0,014
Prolactine	[5,80:11,20] 10,10	[4,00:12,90] 7,40	[5,40:11,20] 5,40	0,310
Total Sperm Number	**	[9030000:23000000] 21,000,000	[112000000:122000000] 112,000,000	< 0,001
VHI-10 Points	[5,00:21,00] 18,00	[2,00:19,50] 7,00	[0,00:18,00] 11,00	0,105
Fundamental frequency F0 Hz(Mean Pitch)	[140,684:196,945] 195,358	[140,684:234,829] 144,634	[140,684:171,669] 171,669	0,693
Jitter % (frequency perturbation jitter)	[0,18:0,40] 0,22	[0,18:0,22] 0,19	[0,29:0,40] 0,29	0,003
Shimmer % (amplitude perturbation shimmer)	[1,414:3,687] 2,817	[1,252:2,817] 2,467	[1,915:3,687] 1,915	0,173
HNR dB	[20,117:29,693] 23,233	[23,233:29,693] 25,048	[20,117:22,649] 22,649	0,005

- For the Jitter % variable, there was a statistically significant difference between Group 2 and Group 3 (p = 0.003).The Jitter % values were higher in Group 3.
- For the HNR dB variable, there was a statistically significant difference between Group 2 and Group 3 (p = 0.008).The HNR dB values were higher in Group 2. The p values between the groups are shown in Table 2.

There was a moderate negative linear correlation between FSH and Fundamental frequency F0 Hz (Mean Pitch) (r=-0.580, p < 0.01). In other words, there was an inverse proportional relationship; as one increased, the other decreased and increased as it decreased. There was a moderately positive linear correlation between FSH and jitter % (r = 0.398, p < 0.01).In other words, there was a direct proportional relationship; as one increased, the other increased and decreased as it decreased.

There was a weak negative linear correlation between prolactin and jitter % (r=-0.290, p < 0.05). That is, there was an inverse proportional relationship; as one increased, the other decreased and increased as it decreased. There was a weak negative linear correlation between prolactin and shimmer % (r=-0.344, p < 0.01). That is, there was an inverse proportional relationship; as one increased, the other decreased and increased as it decreased .There was a weak positive linear correlation between Prolactin and HNR dB (r = 0.290, p < 0.05). In other words, there was a direct proportional relationship; as one increased, the other increased and decreased as it decreased.

Table 3 p and r values

Variables	Fundamental frequency F0 Hz(Mean Pitch))	Jitter % (frequency perturbation jitter)	Shimmer % (amplitude perturbation shimmer)	HNR dB
FSH	-,580**	,398**	-,072	-,141
LH	-,008	-,108	-,072	,121
Prolactine	,036	-,290*	-,344**	,290*
Testosterone	,082	,145	,871**	-,452**

*P < 0.05 , ** P < 0.01, r < 0.2 too weak, 0.2–0.4 weak, 0.4–0.6 moderate intensity, 0.6–0.8 high, 0.8 > very high

There was a very strong positive linear correlation between testosterone and shimmer % (r = 0.871, p < 0.01). In other words, there was a direct proportional relationship; as one increased, the other increased and decreased as it decreased. There was a moderate negative linear correlation between testosterone and HNR dB (r=-0.452, p < 0.01). In other words, there was an inverse proportional correlation;

as one increased, the other decreased and increased as it decreased. It is shown in Table 3 with p and r values.

Discussion

It is thought that the voice and hormones have an effect on the larynx, which is the main structure that creates the voice [5]. The fact that the voice begins to differentiate in puberty, during which the changes in hormonal balance affect many parts of the body, suggests a possible cause for voice change in this pubertal period and shapes the basis of studies and many hypotheses about the effect of hormones on voice. For instance, the changes in estrogen levels in the pubertal period in women have been hypothesized to result in mass and volume increase in the vocal cords by causing laryngeal water retention, edema, and interstitial tissue increase and venous dilatation, and thereby leading to the loss of high voice notes, vocal instability, and fatigue, an uncertain pitch in the voice, decreased vocal efficiency, decreased vocal power, and decreased vocal flexibility.Again, the same study suggested that the decrease in estrogen levels on the 21st day of the cycle causes a perceptual change in the voice [6]. The ratio of testosterone and progesterone was found to be high in men with Reinke's edema. This finding supports the view that hormones affect the larynx mucosa and cause edema [7]. Nacci et al. proposed the hypothesis that there are specific receptors for sex hormones in the larynx. In their study, vocal cord samples from cadavers, healthy people, and patients with laryngeal cancer were examined for the presence of estrogen, progesterone, and androgen receptors using an immunohistochemical method. However, mild estrogen and progesterone receptors were observed in cancerous vocal cord samples, but no receptors were detected in other samples. This study reported that there is no receptor for sex hormones in the larynx [5]. We aimed to examine the vocal characteristics of infertile male patients. In this study, we investigated the effects of LH, FSH, prolactin, and testosterone on voice, whose disorders can cause infertility.

We found a moderate negative linear correlation between FSH and Fundamental frequency F0 Hz (Mean Pitch). (That is, there was an inverse correlation; as one increased, the other decreased and increased as it decreased). We found a moderately positive linear correlation between FSH and jitter %. (That is, there was a direct proportional relationship; as one increased, the other increased and decreased as it decreased). We determined a weak negative linear correlation between prolactin and jitter %. There was a weak negative linear correlation between prolactin and shimmer %.There was a weak positive linear correlation between prolactin and HNR dB. To the best of our knowledge, there is no article in the literature investigating the effect of prolactin on voice. There was a very strong positive linear correlation between testosterone and shimmer %.There was a moderate negative linear correlation between testosterone and HNR dB. Our study also determined a difference in the mean testosterone level between Group 2 and Group 3. Testosterone level was lower in Group 3. However, this difference was not statistically significant. Comparison of Group 2 and Group 3 revealed a higher Jitter % value in Group 3. Again, between these two groups, the HNR dB values were higher in Group 2. Our study demonstrated that when testosterone level is low, jitter frequency increases, and HNR dB values decrease. Again, the comparison of Group 2 and Group 3 revealed a higher f0 value in Group 3 with low mean testosterone.

Pedersen et al. reported that, in pubertal women, the fundamental frequency (f0) in continuing speech decreases as in men, but this decrease is less marked in women. They stated that this situation was caused by estrogen in women and testosterone in men. The difference in the decreasing trend in frequency was attributed to the difference in the effects of estrogen and testosterone [8]. Our study demonstrated that the azoospermic (group 1) group had the highest mean f0 value among the three groups whose testosterone values were in the normal range, but this difference was not statistically significant.

Chae et al. performed voice analysis in patients with Premenstrual syndrome(PMS), characterized by ovarian hormonal changes and depression-like findings different from the typical symptoms before the menstrual cycle. No significant difference was observed in Pms + and PMS (-) group patients in terms of the objective properties of voice. However, among the Pms + patients, the jitter of the voice in the premenstrual phase was significantly higher than the jitter in the follicular phase [9]. The voice analysis in patients receiving HRT (hormone replacement therapy/estrogen) during the menopausal period by Hamdan et al. revealed that habitual pitch value was affected by HRT [10]. Our study on male infertile individuals showed that jitter frequency increased with low testosterone levels.

There are many articles about the effect of testosterone on voice. In order to reveal the effect of testosterone on the voice more clearly, it would be more effective to examine the studies in which testosterone hormone therapy was given to a female patient for any reason. In a case report presented by Cler et al., after a transmasculine individual received testosterone treatment for one year, the f0 value decreased from the female to the male individual's normal range. Furthermore, the patient's regular endoscopic examinations showed that the larynx structure also exhibited the changes seen in a male individual at puberty. (A longer and forward-leaning larynx) [11]. In another study, testosterone was given regularly by a subcutaneous implant in 10 female patients, and voice analyses were performed at 3, 6, and 12 months. No significant change was found in the objective properties of the voice. According to this 12-month study, testosterone hormone therapy did not affect voice quality in female patients. However, it should be noted that the testosterone dose in this study was the therapeutic dose, not the high dose used for transgender individuals [12]. DHEAS and bioavailable testosterone were found to be associated with low-frequency baseline speech f0 in men [13]. Voice analysis in individuals who were given testosterone therapy in the transition from woman to man showed that testosterone decreased the f0 value. In another study, in women treated with testosterone, a significant decrease in the mean pitch value was observed in the high-dose group after the 24th week, in a dose- and concentration-dependent manner [14]. In female patients receiving androgen therapy, f0 and pitch values decreased in 3–4 months of treatment, approaching the range of a normal male individual [15]. In the study of Akcam et al., the f0 value in untreated Idiopathic Hypogonadotropic Hypogonadism (IHH) men had a value between a normal man and woman. As the IHH patient is treated with male androgens, the f0 value approaches that of a normal man. That is, as testosterone increases in the body, the f0 value decreases [16]. Another study suggested that high testosterone was associated with low fundamental frequency (f0) in blood and saliva analysis using the diurnal rhythm of testosterone [17]. A study examining the difference between male and female voices determined the masculine voice f0, body mass index (BMI), length, and testosterone in men to be effective [18]. Contrary to other articles, one study suggests that the main effect on male voice formation is related to testicular volume rather than testosterone concentration [19]. Androgen deprivation therapy (ADT) has effects on the human voice. It was determined that F0, jitter, shimmer, and MPT (maximum phonation time) were affected in patients receiving ADT. Moreover, the satisfaction of the patients with their voices was also negatively affected. However, the limitation of this study was the limited number of patients [20]. Again, the testosterone hormone therapy given to male professional singers was determined to cause a decrease in pitch value, changes in voice quality, and loss of voice control in the short term (1st month), but these improved in the 6th month and were temporary [2]. In a study conducted with elderly male patients, voice parameters did not differ depending on the androgen level. In patients with reduced estrogen levels, a significant increase was detected in mean baseline frequency. This suggests that androgens have no effect on voice in older men [21]. In our study, the mean testosterone levels in Group 2 and Group 3 were different. Testosterone level was lower in Group 3. However, this difference was not statistically significant. Comparison of Group 2 and Group 3 revealed a higher Jitter % value in Group 3. Again, between these two groups HNR dB values were higher in Group 2. Our study showed that when testosterone level is low, jitter frequency increases and HNR dB values decrease. Moreover, the f0 value was higher in group 3 with low mean testosterone compared to Group 2.

A thorough literature review indicated that articles about voice analysis in otorhinolaryngology are limited compared to other titles. Our study is different from the previous literature and is the first study that categorizes infertile men according to spermiogram and hormone levels and performs voice analysis. Besides, studies on voice analysis were mostly performed on women exposed to hormonal cycles, patients receiving therapeutic hormone therapy, transgender individuals, and patients receiving treatment for acromegaly or other hormonal disorders [10–12, 16, 22, 23]. Not only the sex hormones have an effect on the voice. There are also studies investigating the effect of growth hormones. In one study, voice jitter increased in patients with acromegaly (excess growth hormone) without treatment compared to the control group, but the difference was not statistically significant. Moreover, the hoarseness feature of the voice became more evident in the patients [23]. Another study analysing some voice formants used in Brazil showed that patients with a congenital isolated growth hormone deficiency had higher frequencies compared to the control. The reason for this difference was attributed to having smaller oral and pharyngeal cavities due to growth hormone deficiency [24]. Increased Igf-1 in patients with acromegaly has a hypertrophic effect on the vocal cords and surrounding tissue. Mean VHI scores at the time of diagnosis and during the treatment of acromegaly were within normal limits, although they decreased at follow-up. Mucosal edema and hypertrophy were largely resolved during treatment. There was no significant change in objective sound parameters [22]. The study of Andrade et al. argues that semi-occluded vocal tract (SOVTT) and choir training exercises can improve acoustic vocal parameters in patients with growth hormone deficiency [25].

Voice Handicap Index is a self-report questionnaire consisting of 30 items. It has three subgroups, each consisting of 10 items, functional, physical, and emotional. Each item is given a value between 0 and 4 by the patient, with a maximum total score of 120. The higher the score, the bigger the problem with the voice. And it is used as a valuable tool in voice clinical practice and can provide sufficient information to the clinician [4]. Kılıç et al. investigated the reliability and validity of the Turkish version of the Voice Handicap Index. They aimed to develop a short version of the VHI that would provide ease of administration and concluded that the Turkish version of the Voice Handicap Index was more appropriate to use in clinics as some items were problematic [3]. In our study, the highest mean value of VHI-10 was in azoospermic group 1, with a value of 18. This indicates that this group feels the voice problem more. This situation may have caused a change in the voice as a result of the hormones and growth factors examined here, or under psychological and social oppression as the individual was aware of the possibility of having fewer children compared to other groups, and this situation was seen as a weakness in eastern societies. Of course, this could be the subject of a different study.

Conclusions

Our current opinion is that testosterone is more effective on voice, especially in male individuals. Perhaps, instead of a single hormone, it would be more logical to think that many hormones, growth factors, and local factors are effective.

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Data Availability All data and material are in accordance with standards and can be shared.

Declarations

Conflict of interest There is no financial support or conflict of interest in this study.

Statement of Ethics This study protocol was reviewed and approved by XXXX Hospital : University of Health Sciences Adana City Training and Research Hospital Clinical Research Ethics Committee], approval number : 80, Decision Number : 1403, 06.05.2021)

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