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ORIGINAL ARTICLE

Male Health

Evaluation of gonadotropin-replacement therapy in male patients with hypogonadotropic hypogonadism

Mazhar Ortac[1], Muhammed Hidir[2], Emre Salabas[3], Abubekir Boyuk[2], Caner Bese[2], Yasar Pazir[2], Ates Kadioglu[2]

Hypogonadotropic hypogonadism (HH) is a rare disease in which medical treatment has a high success rate to achieve fertility. This study aimed to analyze the efficacy of hormone replacement therapy and determine predictive factors for successful spermatogenesis and spontaneous pregnancy in patients with idiopathic HH. A total of 112 patients with low testosterone (T), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and normal prolactin levels were diagnosed with HH and administered LH and FSH analogs as hormone replacement therapy. During treatment, 96 (85.7%) patients had sperm present in ejaculate samples. Among these patients, 72 were married and wanted a child. Of these 72 patients, 48 (66.7%) of couples had pregnancies from natural conception. After initiation of treatment, the mean time for the appearance of sperm in semen was 9.48 months. There were no significant differences between baseline FSH, T, and LH levels; however, older age, larger testicular size, and low rate of undescended testes were favorable factors for successful spermatogenesis. Larger testicular size and older age were also the main predictive factors for natural conception. We found that patients with undescended testes had a younger age, smaller testes, and lower T levels compared with patients exhibiting descended testes. The rate of sperm found in the ejaculate was not significantly decreased in patients with undescended compared with descended testis (73.7% *vs* 87.6%, $P = 0.261$). The medical approach for males with HH and azoospermia provides a successful treatment modality in regard to successful spermatogenesis and achievement of pregnancy.

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INTRODUCTION

The normal development of fertility needs pulsatile secretion of hypothalamic gonadotropin-releasing hormone (GnRH), which stimulates the synthesis of gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) from the anterior pituitary. Gonadotrophins stimulate gonadal testosterone production and spermatogenesis. LH stimulates testicular Leydig cell proliferation and testosterone secretion, whereas FSH induces the development of spermatogenesis via the activation of sertoli cells.[1,2] Both LH and FSH are the primary hormonal factors regulating testicular functions required for male fertility, via the hypothalamic-pituitary-gonadal (HPG) axis. Failure of this axis, such as disrupted GnRH or gonadotrophin secretion, leads to clinical hypogonadotropic hypogonadism (HH).[3] HH may be either acquired or congenital and is a relatively rare cause (<1%) of male infertility. A congenital form of HH accompanied with anosmia is referred to as Kallmann syndrome.[4]

Regardless of etiology, HH is one of the few causes of male infertility treatable with hormone replacement. Several clinical studies demonstrated that subcutaneous gonadotropin injections or pulsatile GnRH therapy induced the development of secondary sexual characteristics and spermatogenesis, as well as testicular enlargement. Both gonadotropin therapy and GnRH induced the appearance of sperm in the ejaculate.[5–8] Besides gonadotropin therapy, other studies showed that human chorionic gonadotrophin (hCG) treatment without FSH may stimulate spermatogenesis, particularly in patients with a large testicular volume (>4 ml) and no history of cryptorchidism.[9] Moreover, FSH treatment stimulated partial spermatogenesis in a hypogonadal mouse model.[10] In clinical practice, traditional treatment includes the administration of hCG (1000–1500 IU) and FSH (75–150 IU) 2 to 3 times per week.[7] There is no ideal pharmaceutical dosage for HH treatment in the current literature. Furthermore, the success rate of treatment and its influencing factors have not been fully established.[8,9,11,12] Studies on this topic lack the necessary patient population size due to the low incidence of the disease and the heterogeneity of treatment options. A golden standard medical treatment for HH remains to be defined. The aim of this study was to investigate the efficacy of subcutaneous gonadotropin injections and determine predictive factors for successful spermatogenesis and spontaneous pregnancy in 135 patients diagnosed with HH.

PATIENTS AND METHODS

A total of 135 azoospermic patients diagnosed with idiopathic hypogonadotropic hypogonadism (IHH) (2002–2012) were included in this retrospective study. The diagnosis of IHH included physical

[1]Haseki Training and Research Hospital, Department of Urology, Istanbul 34096, Turkey; [2]Department of Urology, Istanbul Faculty of Medicine, Istanbul University, Istanbul 34104, Turkey; [3]Department of Urology, Biruni University Hospital, Istanbul 34295, Turkey.
Correspondence: Dr. M Ortac (m_ortac@hotmail.com)
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examination, low testosterone levels with low/inappropriately normal FSH/LH values, normal prolactin levels, and azoospermia. Anatomical hypophysis pathologies shown by magnetic resonance imaging (MRI) were an exclusion criterion. Patients did not undergo genetic testing for diagnosed Kallmann syndrome or normosmic IHH. Clinical findings including testicle volumes, history of undescended testis, and medical and family histories recorded on enrollment. Average values of both testicular volumes were recorded via a Prader orchidometer (ASSI, Westbury, NY, USA). Semen samples were obtained via masturbation with visual erotic stimulation at the hospital. All semen samples were analyzed in the andrology laboratory of Istanbul Medical Faculty according to the criterion of the World Health Organization (WHO) laboratory manual for the examination and processing of human semen 1999 (before 2010) and 2010 (after 2010).[13,14]

Patients diagnosed with IHH were administered LH and FSH analogs as hormone replacement therapy. A total of 23 patients were excluded from the study due to insufficient medical data. All of the remaining patients were followed up with quarterly semen and hormonal analysis. This study was approved by the Institutional Review Board (IRB) protocols of Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey and informed consent was waived for this retrospective study.

Treatment protocol

Urine-derived gonadotrophin therapy, which had been in use until 2010, was changed to recombinant form. Intramuscular hCG treatment started with a dose of 1500 IU (twice per week) and adjusted according to testosterone levels and testicular development over 6 months. If the patient remained azoospermic at the end of 6 months, FSH treatment (75–150 IU, twice per week) was added to the regime. Target FSH levels of 4–6 IU were achieved by FSH dose adjustments. The follow-up protocol included the quarterly assessment of semen, and FSH and testosterone levels.

The recombinant forms of FSH and LH were Gonal F (Merck-Serono, Geneva, Switzerland) and Ovitrelle (Merck-Serono), respectively. The urine forms of FSH and LH were Menogon (Ferring, GmbH, Kiel, Germany) and Pregnyl (Organon, Oss, Netherlands), respectively.

Statistical analyses

The Statistical Package of Social Sciences (SPSS) for Windows version 20 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. We divided patients into two groups based on the presence of sperm. Thereafter, couples with sperm were divided into two groups according to their pregnancy status. Categorical variables were presented as numbers and percentages and compared using the Chi-square test. Continuous variables were presented as means and standard deviations and compared using the independent sample *t*-test. Dependent variables were examined by paired samples *t*-test. Correlation analyses were evaluated using the Pearson's correlation coefficient. For multivariate analysis, possible factors identified with univariate analyses were further examined by logistic regression analysis to determine independent predictors of patient outcome. Statistical significance was considered when the two-tailed value of $P < 0.05$.

RESULTS

A summary of baseline data is presented in **Table 1**. A total of 112 patients with IHH and azoospermia were investigated, and the average age at diagnosis was 27.9 (range: 15–51) years. The baseline LH, FSH, and testosterone levels were 0.53 ± 0.77 IU l^{-1}, 0.63 ± 0.61 IU l^{-1}, and 1.10 ± 1.90 ng dl^{-1}, respectively. Approximately half of the patients had received various treatment options, such as gonadotropins or androgen replacement, before presenting at our clinic. Fifteen patients (13.4%) had a history of cryptorchidism. Mean baseline testicular size was 5.16 ± 2.43 ml, lower than the normal expected testicular size. Following the combined hormonal treatment regime, 85.7% (96/112) of patients had sperm detected in ejaculate samples.

The mean time for the first appearance of sperm in treated patients was 9.48 ± 6.8 months. The mean FSH and testosterone levels at the time of first sperm detection were 3.29 ± 2.19 IU l^{-1} and 8.27 ± 3.57 ng dl^{-1}, respectively.

The mean serum FSH, LH, and testosterone levels, age, testicular volume, and history of undescended testis were compared between the two groups differentiated by the absence or appearance of sperm in the ejaculate. According to logistic regression and *t*-test analysis, no statistically significant differences were detected between these two groups in terms of baseline FSH, testosterone, and LH (all $P > 0.05$). However, patients with successful spermatogenesis were older (28.63 *vs* 23.75 years, $P = 0.001$), had a larger testicular size (5.02 *vs* 5.15 ml, $P = 0.019$) and a lower rate of undescended testes (0.11 *vs* 0.45, $P = 0.026$) (**Table 2** and **3**). In patients with successful spermatogenesis, the mean testicular volume increased after gonadotropin treatment (5.3 ± 4.0 ml *vs* 11.6 ± 9.0 ml, $P = 0.001$), compared with patients lacking sperm.

Among the patients with induced spermatogenesis, 70 patients wanted children and 48 of these patients impregnated their partners by natural conception and seven required assisted reproductive technology (ART). The mean sperm count was 10.19×10^6 ml^{-1} at the time of spontaneous pregnancy. The mean time needed for spontaneous pregnancy was 21 ± 9 months. According to logistic regression analysis, increased age and larger testicular size were the main predictors factors of spontaneous pregnancy (**Table 4** and **5**). The average age of patients with or without spontaneous pregnancy was 30.0 and 21.2 years, respectively ($P = 0.001$). The average testicular size of patients with or without spontaneous pregnancy was 5.31 ± 2.40 ml and 4.68 ± 1.96 ml ($P = 0.0018$), respectively. However, the mean serum FSH, LH, and testosterone levels, and a history of undescended testis were found

Table 1: Baseline data of patients (*n*=112)

Variables	*Minimum*	*Maximum*	*Mean±s.d.*
Age (year)	15.0	51.0	27.9±6.6
Testicular size (ml)	1.00	11.00	5.16±2.43
FSH baseline (IU l^{-1})	0.04	3.50	0.63±0.61
Testosterone baseline (ng dl^{-1})	0.41	13.00	1.10±1.90
LH baseline (IU l^{-1})	0.23	4.90	0.53±0.77

Normal range: FSH (1.5–12.4 IU l^{-1}); testosterone (2.18–9.06 ng dl^{-1}); LH (1.7–8.6 IU l^{-1}). FSH: follicle-stimulating hormone; LH: luteinizing hormone; s.d.: standard deviation

Table 2: Predictive factors for spermatogenesis (*t*-test)

Variables	*Sperm (+) (n=96)*	*Sperm (–) (n=16)*	*P*
Age (year)	28.6	23.8	0.001*
Cryptorchidism	0.11	0.25	0.026*
Testicular size (ml)	5.15	5.02	0.019*
FSH (IU l^{-1})	0.63	0.59	0.163
Testosterone (ng dl^{-1})	0.71	1.16	0.127
LH (IU l^{-1})	0.55	0.44	0.275

Normal range: FSH (1.5–12.4 IU l^{-1}); testosterone (2.18–9.06 ng dl^{-1}); LH (1.7–8.6 IU l^{-1}). *P<0.05 is defined as statistical significance. Sperm (+): sperm appearance in the ejaculate; Sperm (–): no sperm appearance in the ejaculate LH: luteinizing hormone; FSH: follicle-stimulating hormone

Table 3: Predictive factors for spermatogenesis (correlated regression analysis)

Variables	*B*	*s.e.*	*Wald test statistics*	*df*	*P*	*Exp (B)*	*95% CI for EXP (B)*
Age (year)	0.121	0.057	4.458	1	0.025*	1.128	0.109–1.562
Cryptorchidism	1.170	0.552	4.484	1	0.024*	3.221	0.973–2.283
Testicular size (ml)	2.518	1.080	5.439	1	0.020*	12.402	1.034–4.239
FSH baseline (IU l^{-1})	0.109	0.598	0.033	1	0.856	1.115	0.096–1.596
Testosterone baseline (ng dl^{-1})	0.051	0.217	0.056	1	0.812	1.053	0.009–1.609
LH baseline (IU l^{-1})	0.115	0.560	0.042	1	0.837	1.122	0.105–1.360

*$P<0.05$ is defined as statistical significance. Normal range: FSH (1.5–12.4 IU l^{-1}); testosterone: (2.18–9.06 ng dl^{-1}); LH: (1.7–8.6 IU l^{-1}). *B*: coefficient; s.e.: standard error; df: degree of freedom; Exp (*B*): odds ratio; CI: confidence interval; LH: luteinizing hormone; FSH: follicle-stimulating hormone

Table 4: Predictive factors for pregnancy (*t*-test)

Variables	*Pregnancy (+) (n=48)*	*Pregnancy (–) (n=22)*	*P*
Age (year)	30.04	21.22	0.001*
Cryptorchidism	0.06	0.27	0.172
Testicular size (ml)	5.31	4.68	0.018*
FSH (IU l^{-1})	0.53	0.67	0.237
Testosterone (ng dl^{-1})	1.36	0.73	0.184
LH (IU l^{-1})	0.37	0.59	0.155

*$P<0.05$ is defined as statistical significance. Pregnancy (+): presence intrauterine gestational sac; Pregnancy (–): no intrauterine gestational sac. Normal range: FSH (1.5–12.4 IU l^{-1}); testosterone (2.18–9.06 ng dl^{-1}); LH (1.7–8.6 IU l^{-1}). LH: luteinizing hormone; FSH: follicle-stimulating hormone

to have no statistically significant differences between the two groups (all $P > 0.05$).

Subgroup analyses were used to investigate whether undescended testes were a negative predictor factor for the outcome of therapy. We found that patients with undescended testes had a younger age, smaller testes, and lower testosterone level compared with patients with descended testes (all $P < 0.05$) (**Table 6**). In addition, the rate of sperm found in the ejaculate was decreased in patients with undescended testis; however, the difference was not statistically significant (73.7% *vs* 87.6%, $P = 0.261$). Among the treated cryptorchid patients with induced spermatogenesis ($n = 11$), of 9 patients wanting children, only four impregnated their partners with natural conception. In safety evaluation, no patients developed severe effects during the treatment period.

DISCUSSION

The LH-FSH combination is an effective therapy for restoring spermatogenesis in male patients with gonadotrophin-deficiency.[2] In this retrospective study, we investigated the efficacy and safety of gonadotropin therapy for the restoration of spermatogenesis in 112 HH patients. In addition, the potential effect of baseline factors on treatment outcomes was also explored. Approximately 85.7% (96/112) of the patients had detectable sperm in ejaculates during treatment. Dwyer *et al.*[15] and the European Metrodin HP Study Group[16] reported that FSH/hCG treatment induced spermatogenesis in 84% and 89.3% of patients with HH, respectively. In a meta-analysis of 30 studies by Rastrelli *et al.*,[8] the overall success rate of gonadotropin therapy on gonadotrophin-deficient male patients was 75% (range: 69%–81%).

Treatment of HH may take a long time to induce successful spermatogenesis. Dwyer *et al.*[15] reported that the time to develop spermatogenesis ranges from 3 to 19 months and 9 to 12 months for mono and combined gonadotrophin treatments, respectively. Different time periods for stimulation of sperm production may reflect different population groups and treatment regimens.[15] In this study, the mean time to achieve spermatogenesis was 9.4 months, consistent with other studies reporting the median time of the first appearance of sperm appearance reported as 9–12 months.[16–20] Matsumoto *et al.*[19] found that the median time of first sperm appearance was 12.9 (range: 6.1–17.1) months. However, Liu *et al.*[21] reported a longer median time of 15 months to obtain sperm in the ejaculate.[16] They speculated that this difference may relate to longer treatment sessions with lower dose hCG treatment and longer follow-up time.

Several studies have focused on the predictive factors for successful spermatogenesis in patients with HH.[6,9,12,22,23] In the current study, larger basal testicular volume, older age, and a lower incidence rate of undescended testis were positive predictive factors for achieving sperm in the ejaculate. Testicular size increased significantly after gonadotrophin therapy in patients with induced spermatogenesis. These findings support previous studies in which basal testicular volume and the rate of undescended testis were reported as the main prognostic factors for the restoration of spermatogenesis.[2] The relationship between testicular development and sperm restoration is expected since it is known that normal testicular function is an essential part of a functional HPG axis in healthy adult men.[3] It was proposed that mean testicular volumes >4 ml defined an important threshold for the success of sperm restoration.[24] Spratt *et al.*[25] reported that patients with partial/normal nocturnal LH pulses had larger testicular volume (4 ml) compared with those without LH pulses. Furthermore, Burris *et al.*[12] found that testicular sizes <4 ml were associated with complete gonadotropin deficiency, whereas a size >4 ml suggested partial gonadotropin deficiency. Although 71% of HH patients with a testicular size of ≥4 ml responded to gonadotrophin treatment, this rate was only 36% in patients with a testicular size of <4 ml.[26]

In the current study, testicular size was measured via the Prader orchidometer. Despite this subjective method, the orchidometer is closely correlated with measurements by ultrasonography. However, it should be noted that the orchidometer was shown to overestimate testicular volume, particularly with small testes.[27] Cryptorchidism is a common anomaly of male genitalia, estimated to affect 2%–4% of full-term male infants, and is a significant prognostic factor for fertility in both the general population and HH patients. A history of undescended testes almost always negatively affects the restoration of sperm in patients with HH.[9,15,17] In this study, we found that HH patients with cryptorchidism had a lower probability of achieving spermatogenesis. Furthermore, patients with undescended testes had smaller testicular volumes compared with descended testes in HH patients, possibly explaining the poor induction of spermatogenesis in cryptorchid testes. Similarly, in a study of 223 HH patients by Liu *et al.*,[21] the sperm restoration rate was only 64%, and an association was found between basal testicular volume and the presence of undescended testicles with the restoration of sperm. Pitteloud *et al.*[22] reported a high incidence of cryptorchidism (40%) in patients with HH that was shown to be a negative predictive factor for both testicular growth and spermatogenesis.

Although age is not a factor for successful spermatogenesis in the current literature, older patients had better outcomes compared with

Table 5: Predictive factors for pregnancy (correlated regression analysis)

Variables	*B*	*s.e.*	*Wald test statistics*	*df*	*P*	*Exp (B)*	*95% CI for EXP (B)*
Age (year)	0.849	0.298	8.120	1	0.004*	2.337	1.303–4.191
Cryptorchidism	1.306	1.700	0.591	1	0.442	0.271	0.010–7.575
Testicular size (ml)	0.985	0.459	4.598	1	0.022*	2.677	1.088–6.586
FSH baseline (IU l^{-1})	1.225	2.160	0.322	1	0.571	3.405	0.049–2.346
Testosterone baseline (ng dl^{-1})	0.346	0.331	1.092	1	0.296	0.708	0.370–1.354
LH baseline (IU l^{-1})	0.846	1.168	0.524	1	0.469	0.429	0.043–4.240

*$P<0.05$ is defined as statistical significance. Normal range: FSH (1.5–12.4 IU l^{-1}); testosterone (2.18–9.06 ng dl^{-1}); LH (1.7–8.6 IU l^{-1}). *B*: coefficient; s.e.: standard error; df: degree of freedom; Exp (*B*): odds ratio; CI: confidence interval; LH: luteinizing hormone; FSH: follicle-stimulating hormone

Table 6: Comparable patients with descended and undescended testes

Variables	*n*	*Mean*	*s.d.*	*P*
Age (year)				
Descended testes	97	28.8	6.4	0.001*
Cryptorchidism	15	22.5	5.6	
FSH (IU l^{-1})				
Descended testes	97	0.643	0.628	0.418
Cryptorchidism	15	0.505	0.501	
Testosterone (ng dl^{-1})				
Descended testes	97	1.210	2.013	0.001*
Cryptorchidism	15	0.382	0.565	
LH (IU l^{-1})				
Descended testes	97	0.555	0.788	0.388
Cryptorchidism	15	0.370	0.634	
Testicular size (ml)				
Descended testes	97	5.474	2.389	0*
Cryptorchidism	15	3.133	1.597	

Normal range: FSH (1.5–12.4 IU l^{-1}); testosterone (2.18–9.06 ng dl^{-1}); LH (1.7–8.6 IU l^{-1}). *$P<0.05$ is defined as statistical significance. LH: luteinizing hormone; FSH: follicle-stimulating hormone; s.d.: standard deviation

younger patients in our study. Liu *et al*.[21] also reported that older age was associated with earlier spermatogenesis in patients with HH. The higher motivation to achieve fertility and thus better compliance with the treatment was suggested as a possible reason for this association.

Regardless of the treatment options, the total number of sperm in patients with IHH generally remains below the normal level. However, this finding does not prevent these patients from become fathers, as pregnancy rates of 50%–80% can be achieved with a sperm concentration of 5×10^6 ml^{-1}.[28,29] Our data showed that nearly 68% of patients who wanted children achieved spontaneous pregnancy with gonadotrophin therapy. Furthermore, testicular volume was the most important predictor for spontaneous pregnancy. According to the existing data, the main predictive factor for spontaneous pregnancy is testicular size.[30] Farhat *et al*.[31] and Buchter *et al*.[32] reported that 58% and 72% of patients, respectively, achieved spontaneous pregnancy with gonadotrophins or GnRH treatment. Both studies also reported that patients with larger testicular size had a better outcome regarding spontaneous pregnancy rates.

In the present work, the average sperm concentration was 10×10^6 ml^{-1} at the time of impregnation and the mean time to achieve spontaneous pregnancy was 21 months, which is agreement with the limited published data. A median treatment duration of 20.5, 20, and 23 months was found by Liu *et al*.,[17] Kung *et al*.,[33] and Burris *et al*.,[12] respectively. However, Liu *et al*.[17] also showed that the median sperm concentration with spontaneous pregnancy was 8.0×10^6 ml^{-1} and the median time to achieve spontaneous pregnancy was 28.2 months. This may be related to the long follow-up time for patients with restored spermatogenesis in the later study. Consequently, these results show that most patients with IHH who undergo gonadotropin treatment may have a child; however, treatment sessions may be prolonged.

In this study, there were no clear data for patients who had received androgen therapy before gonadotropin treatment. According to the current literature, there is no evidence that testosterone replacement treatment prior gonadotropins has an effect on fertility outcomes in patients with IHH. A single retrospective study found that prior testosterone therapy provided a poor prognostic factor for fertility outcomes.[17] However, the meta-analysis published by Rastrelli *et al*.[8] did not support the findings of that study.

In this study, patients did not undergo genetic testing for diagnosed Kallmann syndrome or normosmic IHH. Central HH may be associated with genetic mutation, as nearly 40% of patients typically have some genetic form of hypogonadism. Some mutation may be associated with partial or reversible GnRH deficiency, such as G protein-coupled receptor (*GPR54*) mutations.[34] Treatment-induced increases in total testosterone can be influenced by estradiol levels values, which were not assessed in our study, highlighting a potential limitation.

CONCLUSION

The current medical approach for males with HH and azoospermia allows the successful induction of spermatogenesis for the achievement of pregnancy. Larger testicular volumes of descended testes and older age are positive predictive factors for successful spermatogenesis. Larger testicular volumes and older age also are positive factors for natural conception. Randomized controlled trials with greater patient numbers will be needed for future studies.

AUTHOR CONTRIBUTIONS

MO and AK contributed to the conception and design of the study. AB, YP, and CB acquired the data. MO, ES, and MH drafted the manuscript. MO and AK revised it for intellectual content. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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