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ORIGINAL RESEARCH

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# Testicular sperm retrieval and intracytoplasmic sperm injection outcomes in hypogonadotropic hypogonadal men who remained azoospermic after gonadotropin therapy

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## Abstract

**Background**: Limited data exist regarding the sperm retrieval rate through testicular sperm extraction (TESE) in hypogonadotropic hypogonadal azoospermic men, as well as the fertilization and pregnancy outcomes associated with intracytoplasmic sperm injection (ICSI) using sperm retrieved. The objective of this study was to evaluate sperm retrieval rates through microscopic TESE and to analyze the outcomes of ICSI in hypogonadotropic hypogonadal men who remained azoospermic following gonadotropin therapy. **Methods**: We conducted a retrospective evaluation of microscopic TESE and ICSI outcomes in hypogonadotropic hypogonadal patients who remained azoospermic after gonadotropin therapy between 2004 and 2024. **Results**: Sperm was successfully retrieved from 14 out of 17 patients (82.4%) after gonadotropin treatment. However, no statistically significant differences were found between patients with successful sperm retrieval and those without. ICSI outcomes were performed using frozen-thawed sperm in 12 of the 14 patients, while 2 patients did not proceed with ICSI outcomes. The mean age of the partners was 27 $\pm$ 7.1 years. A total of 25 high-quality embryos were transferred across 18 transfer cycles, yielding an implantation rate of 28%. Six cycles resulted in clinical pregnancy, and four of these pregnancies resulted in live births. **Conclusions**: Gonadotropin therapy can successfully reverse azoospermia in most hypogonadotrophic hypogonadal male patients. For those who remain azoospermic after gonadotropin treatment, testicular sperm can still be retrieved through microscopic TESE, offering a viable option for assisted reproductive technology and the potential for achieving live births.

## Keywords

Infertility; Azoospermia; Hypogonadotropic hypogonadism; Testicular sperm extraction; Intracytoplasmic sperm injection


  

# Extracción de espermatozoides testiculares y resultados de la inyección intracitoplasmática de espermatozoides en hombres con hipogonadismo hipogonadotrófico que permanecieron azospérmicos después de la terapia con gonadotropinas


## Resumen

**Antecedentes**: Existen datos limitados sobre la tasa de recuperación de espermatozoides mediante la extracción testicular de esperma (TESE) en hombres azoospérmicos con hipogonadismo hipogonadotrópico, así como sobre los resultados de fertilización y embarazo asociados a la inyección intracitoplasmática de espermatozoides (ICSI) utilizando los espermatozoides recuperados. El objetivo de este estudio fue evaluar las tasas de recuperación de espermatozoides mediante TESE microscópica y analizar los resultados de la ICSI en hombres con hipogonadismo hipogonadotrópico que permanecieron azoospérmicos tras la terapia con gonadotropinas. **Métodos**: Se realizó una evaluación retrospectiva de los resultados de TESE microscópica e ICSI en pacientes con hipogonadismo hipogonadotrópico que permanecieron azoospérmicos después de la terapia con gonadotropinas entre 2004 y 2024. **Resultados**: Se recuperaron espermatozoides en 14 de los 17 pacientes (82.4%) tras el tratamiento con gonadotropinas. Sin embargo, no se encontraron diferencias estadísticamente significativas entre los pacientes con recuperación exitosa de espermatozoides y aquellos sin ella. Los resultados de la ICSI se realizaron utilizando espermatozoides congelados-descongelados en 12 de los 14 pacientes, mientras que 2 pacientes no continuaron con ICSI. La edad media de las parejas fue de 27 $\pm$ 7.1 años. Se transfirieron un total de 25 embriones de alta calidad en 18 ciclos de transferencia, con una tasa de implantación del 28%. Seis ciclos resultaron en embarazo clínico, y cuatro de estos embarazos culminaron en nacimientos vivos. **Conclusiones**: La terapia con gonadotropinas puede revertir con éxito la azoospermia en la mayoría de los pacientes varones con hipogonadismo hipogonadotrópico. Para aquellos que permanecen azoospérmicos tras el tratamiento con gonadotropinas, aún es posible recuperar espermatozoides testiculares mediante TESE microscópica, ofreciendo una opción viable dentro de la tecnología de reproducción asistida y la posibilidad de lograr nacimientos vivos.



## Palabras Clave

Infertilidad; Azoospermia; Hipogonadismo hipogonadotrópico; Extracción testicular de esperma; Inyección intracitoplasmática de espermatozoides


## 1. Introduction

Male hypogonadotropic hypogonadism (HH) arises from either congenital causes, such as Kallmann syndrome and idiopathic hypogonadotropic hypogonadism, or acquired conditions, including hypothalamic and pituitary tumors, empty sella syndrome, granulomatous disease, hemochromatosis, obesity, anabolic steroid use, and aging. These conditions result in either the absence or insufficiency of hypothalamic gonadotropin-releasing hormone (GnRH) secretion or a failure in pituitary gonadotropin release [1–3]. This rare condition, characterized by minimal testosterone activity and impaired spermatogenesis, represents a treatable cause of non-obstructive azoospermia [3]. In up to 90% of this patient population, gonadotropin replacement therapy with human chorionic gonadotropin (hCG) alone or in combination with follicle-stimulating hormone (FSH) has been shown to restore fertility by stimulating spermatogenesis [4]. In azoospermic men who do not respond to gonadotropin therapy or in those whose partners have diminished ovarian reserve, the use of intracytoplasmic sperm injection (ICSI) with sperm surgically retrieved from the testicles may lead to favorable fertility outcomes [5].

The objective of this study was to evaluate our sperm retrieval rate (SRR) by microscopic testicular sperm extraction (TESE) and to analyze ICSI outcomes in hypogonadotropic hypogonadal men who remained azoospermic after gonadotropin therapy.

## 2. Materials and methods

### 2.1 Patients

We retrospectively evaluated the microscopic TESE and ICSI outcomes of hypogonadotropic hypogonadal patients who remained azoospermic after gonadotropin therapy between 2004 and 2024. Patients were eligible if they had hormonal findings consistent with hypogonadotropic hypogonadism, available pituitary magnetic resonance imaging (MRI) to rule out hypothalamic or pituitary abnormalities, and persistent azoospermia despite receiving gonadotropin therapy for at least six months. The study received approval from our institutional review board.

### 2.2 Clinical and laboratory evaluation

All patients underwent a comprehensive assessment, including a detailed medical history and a thorough physical examination. Azoospermia was confirmed by at least two semen analyses. Seminal parameters were assessed according to the criteria established by the World Health Organization (WHO), with azoospermia defined as the complete absence of spermatozoa following centrifugation of the semen at 3000g for 15 minutes and a thorough examination of the pellet [6].

HH was defined as low morning total testosterone in conjunction with low or inappropriately normal FSH and luteinizing hormone (LH) levels. In borderline cases, free testosterone was calculated using sex hormone-binding globulin and albu-

min levels. Pituitary MRI was performed in all cases to identify any structural lesions that might present and contribute to HH [7].

## 2.3 Treatment protocol

Therapeutic intervention was initiated with either urinary hCG (Pregnyl 5000 IU) or recombinant hCG (Ovitrelle 250 $\mu$g), administered subcutaneously twice weekly, corresponding to a total weekly dose of approximately 3000–5000 IU. Treatment was maintained for 24 weeks, during which patients were monitored every three months with semen analysis and hormonal profiling, and the dose was titrated as needed to achieve optimal serum testosterone levels. Semen samples were obtained via masturbation following 3–7 days of sexual abstinence. In cases where spermatogenesis was not restored with hCG monotherapy, highly purified human menopausal gonadotropin (hMG) (Menopur 75 IU) was subsequently added to the regimen three times per week. During combined gonadotropin therapy, the frequency of hCG administration and the dosage of hMG were gradually adjusted based on periodic hormone measurements. Treatment was continued until an adequate therapeutic response was achieved, with patients counseled that the appearance of spermatozoa in the ejaculate typically required 6 to 12 months of gonadotropin therapy.

## 2.4 Microscopic TESE procedure

Microscopic TESE was performed under sedoanalgesia and local anaesthesia according to the technique previously described by Schlegel [8]. Informed consent was obtained from all patients prior to the procedures. The obtained seminiferous tubule samples were transferred to a sterile Petri dish containing 3 mL of HEPES buffered medium and dissected according to the mechanical technique [9]. Microscopic examination of the wet preparation performed by experienced embryologists was carried out at $\times$400 magnification under an inverted microscope (Olympus IX71, Olympus Corporation, Tokyo, Japan). In cases where sperm could not be retrieved from the dissected testis, the same procedure was applied to the contralateral testis.

In cases where sperm was retrieved, the final sample was combined with the same volume of sperm freezing medium (CryoSperm, Origio, Måløv, Denmark). The resulting mixture was then immersed in liquid nitrogen for storage at −196 °C.

In all patients, a sample of seminiferous tubules was immersed in Bouin's solution and subsequently sent for histopathological evaluation.

## 2.5 Ovarian stimulation and ICSI

Controlled ovarian hyperstimulation was conducted using either the long protocol or the antagonist protocol, as previously described [10]. Oocyte retrieval was performed 36 hours after hCG administration. Following denudation, metaphase II (MII) oocytes were prepared for ICSI procedure. Routine ICSI was performed after a 2 to 2.5-hour incubation period, as previously described by Palermo *et al.* [11]. After 16 to 18 hours, embryos were assessed for fertilization and subsequently for cleavage. Daily evaluations of embryo cleavage were conducted until the transfer day. Embryos were graded according to the grading system of ALPHA Scientists Special Interest Group [12]. Embryo transfers were performed 3 to 5 days post-ICSI, following four to six days of luteal support in the frozen embryo transfer cycle.

## 2.6 Data interpretation

The patients were categorized into two groups based on the presence of spermatozoa during micro-TESE. Group I comprised patients from whom spermatozoa were successfully retrieved, while Group II consisted of patients from whom no spermatozoa could be obtained. Age, HH etiology, ejaculate volume, serum hormone levels at the start of the treatment, type and duration of gonadotropin treatment prior to micro-TESE, the laterality of the micro-TESE procedure (unilateral and bilateral) and the results of histopathological evaluations were compared between groups.

To evaluate the outcomes of ICSI, we assessed the fertilization rate (FR), implantation rate (IR), clinical pregnancy fertilized embryo rate (CPR), and live birth rate (LBR) for those who underwent the procedure. FR is defined as the ICSI. IR was calculated as the percentage of s (mean number of 2 pronuclear embryos) relative to the total number of MII oocytes that underwent he number of gestational sacs observed in the uterine cavity per transferred embryo. CPR was defined as the presence of a fetal heartbeat at least two weeks following the hCG test. Live birth was defined as the delivery of a baby after 24 weeks of gestation. The cumulative CPR refers to the pregnancy rate across both fresh and frozen-thawed embryo transfer (ET) cycles.

## 2.7 Statistical analysis

Kolmogorov-Smirnov test or Shapiro-Wilk test was used to assess the normality of continuous variable distributions. Normally distributed continuous variables were presented as mean $\pm$ standard deviation, while non-normally distributed continuous variables were reported as median. Depending on their distribution, continuous variables were compared using either Student's *t*-test for parametric data or the Mann-Whitney U test for non-parametric data. The categorical variables between the groups were analyzed by using the Chi square test or Fisher's Exact test. Statistical significance was defined as a *p*-value of less than 0.05. Statistical analysis was conducted using Statistical Package for the Social Science (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA).

# 3. Results

## 3.1 Patient characteristics

There were 17 patients who became azoospermic after gonadotropin treatment due to HH. Only one of these patients had HH secondary to pituitary adenoma surgery, while the others had isolated HH. Baseline characteristics of patients with a mean age of 34.4 $\pm$ 5.2 years are summarized in Table 1.

While 10 patients received urinary HCG-based treatment, 7 patients received recombinant HCG-based treatment. There was no statistically significant difference between the treat-

**TABLE 1. Demographic characteristics, initial hormone analyses and treatment protocols of the patients and details of the testicular sperm extraction procedures.**

| | Sperm (+) | Sperm (−) | Total | *p* value |
|---|---|---|---|---|
| Total | 14 (82.4) | 3 (17.6) | 17 (100) | |
| Age (yr) | 35.3 ± 5.1 | 30 ± 3.5 | 34.4 ± 5.2 | 0.112 |
| Testis volume (mL) | 8.6 ± 5.0 | 4.7 ± 3.1 | 7.9 ± 4.9 | 0.218 |
| Ejaculate volume (mL) | 1.8 ± 0.9 | 1.9 ± 0.2 | 1.8 ± 0.8 | 0.791 |
| HH etiology | | | | |
| Congenital | 13 (76.5) | 3 (76.5) | 16 (94.1) | 1.000 |
| Acquired | 1 (5.9) | 0 (0.0) | 1 (5.9) | |
| Hormone profile | | | | |
| FSH (mIU/mL) | 0.42 ± 0.49 | 0.04 ± 0.07 | 0.35 ± 0.47 | 0.091 |
| LH (mIU/mL) | 0.14 ± 0.18 | 0.07 ± 0.00 | 0.13 ± 0.16 | 0.591 |
| Testosterone (ng/mL) | 0.69 ± 0.42 | 1.18 ± 0.53 | 0.78 ± 0.46 | 0.096 |
| Gonadotropin treatment | | | | |
| Type | | | | |
| u-hCG + hMG | 9 (52.9) | 1 (5.9) | 10 (58.8) | 0.537 |
| r-hCG + hMG | 5 (29.4) | 2 (11.8) | 7 (41.2) | |
| Duration (mon) | 16.8 ± 8.2 | 14.0 ± 3.5 | 16.3 ± 7.6 | 0.859 |
| TESE side | | | | |
| Unilateral | 5 (29.4) | 0 (0.0) | 5 (29.4) | 0.515 |
| Bilateral | 9 (52.9) | 3 (17.6) | 12 (70.6) | |
| Histopathology | | | | |
| Incomplete MA | 6 (35.3) | 2 (11.8) | 8 (47.1) | 0.576 |
| Complete MA | 8 (47.0) | 1 (5.9) | 9 (52.9) | |

*Values are presented as number (%) for categorical variables or mean ± standard deviation for continuous variables. HH: hypogonadotropic hypogonadism; FSH: follicle-stimulating hormone; LH: luteinizing hormone; u-hCG: urinary human chorionic gonadotropin; hMG: human menopausal gonadotropin; r-hCG: recombinant human chorionic gonadotropin; TESE: testicular sperm extraction; MA: maturation arrest.*

ment types and treatment durations of the patients (Table 1).

Unilateral micro-TESE was applied to 5 patients and bilateral microscopic TESE was applied to 12 patients, there was no statistical difference between the groups ($p = 0.515$).

### 3.2 Micro-TESE results

While sperm was obtained from 14 patients (82.4%) after gonadotropin treatment, there was no statistically significant difference in terms of the interpreted data between the group in which sperm was obtained and the group in which it was not obtained (Table 1).

Histopathological examination revealed varying degrees of maturation arrest in all patients (Table 1).

### 3.3 ICSI, embryological and clinical outcomes

ICSI was performed using frozen-thawed sperm in 12 of these 14 patients from whom sperm was obtained surgically. The remaining 2 patients did not start ICSI treatment. The mean age of the partners was 27 ± 7.1 years. A total of 25 good quality embryos were transferred in 18 transfer cycles in these patients. The implantation rate was calculated as 28%. Six of these cycles resulted in clinical pregnancy, and four of them resulted in delivery. Three of the pregnancies were singletons and one was twins. Embryological and clinical results are summarized in Table 2 (Ref. [5, 13–17]).

## 4. Discussion

In treating azoospermic men with HH, GnRH or hCG, with or without hMG or FSH, can effectively initiate spermatogenesis. While various protocols have been employed for the treatment of HH, prolonged continuous therapy, along with regular monitoring of hormone levels and semen analyses, is essential for achieving successful sperm production. Typically, the treatment protocols begin with hCG, and once serum testosterone levels stabilize, hMG or FSH is subsequently added to the treatment regimen [2]. The effectiveness of recombinant FSH is comparable to that of urinary FSH [18]. Although the response to gonadotropin treatment varies among patients, spontaneous pregnancy may occur within 6 to 9 months of initiating treatment, with this period potentially extending up to two years [2]. For individuals with persistent azoosper-

**TABLE 2. Results for all cases of testicular sperm extraction and intracytoplasmic sperm injection cycles.**

| | Meseguer *et al.* [13] 2004 | Fahmy *et al.* [14] 2004 | Bakircioglu *et al.* [15] 2007 | Akarsu *et al.* [16] 2009 | Frapsauce *et al.* [17] 2011 | Chen *et al.* [5] 2021 | Present study |
|---|---|---|---|---|---|---|---|
| Treatment duration (mon) | 12 | >6 | 9 | 13 (10–17)* | 20 | 12.1 (6–23)* | 16.3 (6–26)* |
| Sperm extraction method | cTESE | cTESE | mTESE | mTESE | N/R | mTESE | mTESE |
| Sperm retrieval rate (%) | 1/1 (100.0) | 11/15 (73.0) | 2/2 (100.0) | 4/4 (100.0) | 1/1 (100.0) | 8/9 (90.0) | 14/17 (82.4) |
| Sperm for ICSI | Thawed | Fresh/Thawed | Thawed | Fresh/Thawed | Thawed | Fresh | Thawed |
| Oocytes for ICSI | Fresh | Fresh | Fresh | Fresh | Fresh/Thawed | Fresh/Thawed | Fresh |
| No. of oocytes fertilized/retrieved | 4/9 | 53/127 | N/R | 43/95 | 4/7 | 94/156 | 78/155 |
| Fertilization rate (%) | 44.4 | 41.7 | N/R | 45.3 | 57.1 | 60.3 | 50.3 |
| Clinical pregnancy rate/cycle (%) | 0/1 (0.0) | 3/17 (17.6) | N/R | 3/6 (50.0) | 1/2 (50.0) | 6/11 (54.5) | 6/18 (33.3) |
| Cumulative live-birth rate (%) | 0 (0.0) | N/R | N/R | 2/4 (50.0) | 1/1 (100.0) | 5/9 (55.6) | 4/12 (33.3) |

**mean treatment period (treatment range). cTESE: conventional testicular sperm extraction; mTESE: microscopic testicular sperm extraction; ICSI: intracytoplasmic sperm injection; N/R: Not reported.*

mia who do not respond to gonadotropin therapy, as well as azoospermic men with female partners demonstrating reduced ovarian reserve, early intervention with assisted reproductive technology, including surgical sperm retrieval from the testes, may offer favorable fertility outcomes [5].

Limited data exist regarding the sperm recovery rate through TESE in hypogonadotropic hypogonadal azoospermic men, as well as the fertilization and pregnancy outcomes associated with ICSI using sperm retrieved via this method [5, 13–17]. The SRR using the TESE procedure after gonadotropin treatment in men with hypogonadotropic hypogonadal azoospermia has been reported in the literature to range from 73% to 100%. Although all gonadotropin treatments in the literature are based on hCG, they exhibit variations. The common approach, as seen in our study, involves the combination of hCG with hMG [5, 14, 16]. The duration of gonadotropin treatment in these studies varies between 6 and 23 months. In our study, which includes 17 patients, we observed a SRR of 82.4% after an average of 16.3 (range, 6–26) months of gonadotropin treatment. This study is, to our knowledge, the largest series reported to date (Table 2).

ICSI is the only available treatment option for patients with HH who undergo sperm retrieval via TESE. FR in this patient population using ICSI ranges from 41.7% to 60.3%. The CPR per cycle has been reported to vary between 0% and 54.5% [5, 13, 14, 16, 17]. In our study, the FR and CPR per cycle were 50.3% and 33.3%, respectively. Notably, to the best of our knowledge, the LBR we achieved based on a cohort of 12 patients, the largest reported to date was 33.3% (Table 2).

The primary limitations of our study include its retrospective design, the small sample size, and the rarity of this patient population in the literature, as most azoospermic men with HH generally respond to gonadotropin therapy.

## 5. Conclusions

Gonadotropin therapy has the potential to reverse azoospermia in a significant proportion of males with HH. In men who remain azoospermic after gonadotropin treatment, testicular sperm can often be retrieved via micro-TESE in nearly all patients for use in assisted reproductive technology to achieve live births.

### AVAILABILITY OF DATA AND MATERIALS

The data used to support the findings of this study are available from the corresponding author on reasonable request.

### AUTHOR CONTRIBUTIONS

CO—Conception and design. CO, MVK, DAY—Data acquisition. CO, DAY, ES—Data analysis and interpretation. CO, DAY—Drafting manuscript. CO, MVK, DAY, ES, MRG—Critical revision of the manuscript for scientific and factual content; Supervision. CO, MRG—Statistical analysis. All authors read and approved the final manuscript.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study protocol was reviewed and approved by the institutional review board of Baskent University Hospital (Reg. No. KA 24/368). Written informed consent was obtained from all participants included in the study in accordance with ethical standards and the Declaration of Helsinki.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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