Testicular Sperm Extraction (TESE) and Intracytoplasmic Sperm Injection (ICSI) in Hypogonadotropic Hypogonadism with Persistent Azoospermia After Hormonal Therapy

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Purpose: We aimed to retrieve testicular sperm to be employed on intracytoplasmic sperm injection (ICSI) cycles on a male affected of hypogonadotropic hypogonadism (HH) that remained azoospermic after long-time hormonal treatment.

Methods: *Design.* We initially performed hormonal therapy using gonadotropins to achieve spermatogenesis. After several semen analyses, we weighed the possibility of looking for testicular spermatozoa for ICSI. *Setting.* A private university-affiliated setting. *Patient.* A 30-years-old man diagnosed 10 years ago to suffer from idiopathic, prepubertal HH. *Interventions.* Gonadotrophin treatment was initiated with hCG and follicle stimulating hormone (FSH). Testicular sperm extraction was carried out when repeated spermiograms were negative. Motile testicular spermatozoa were cryopreserved and were subsequently employed for ICSI. Multiple follicular development was stimulated with gonadotropins after a downregulation with gonadotropin-releasing hormone (GnRH) antagonists in the woman. *Main Outcome Measures.* Seminal analyses were performed after 3, 6, and 12 months of treatment and serum FSH, luteinizing hormone (LH) and testosterone levels were also measured.

Results: Seminal analysis showed always azoospermia. Serum FSH was 2.9 mIU/mL, serum LH >1 mIU/mL and serum testosterone 7.9 ng/mL (12 months after treatment). Nine oocytes were collected by ultrasound-guided transvaginal route and eight of them were microinjected with motile, frozen-thawed testicular spermatozoa. Four oocytes were fertilized. Three embryos were transferred without pregnancy.

Conclusions: The case report here presented shows that the currently available assisted reproduction techniques may be of value in patients with HH not responding to conventional hormonal treatments.

KEY WORDS: Hypogonadotropic hypogonadism; azoospermia; TESE; ICSI; hormonal therapy.

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INTRODUCTION

Male hypogonadotropic hypogonadism (HH) results either from the abnormal production or secretion of hypothalamic gonadotropin-releasing hormone (GnRH) with consequent diminished pituitary stimulation or from a variety of intrinsic pituitary

abnormalities. Although HH is infrequently diagnosed among infertile males, accounting for less than 1% of cases, it is one of the few causes of nonobstructive azoospermia which can be successfully treated (1,2).

Hormonal therapy using gonadotropins or pulsated GnRH has been reported to be successful in inducing and maintaining spermatogenesis in nearly 90% of patients with primary, complete isolated HH (3,4). Failure in achieving spermatogenesis has been addressed to associated conditions, such as cryptorchidism or primary testicular failure (5). For patients not responding to hormonal treatment, artificial insemination with donor sperm, adoption, or remaining childless were the only alternatives until recently.

Nevertheless, the possibility to use testicular spermatozoa for intracytoplasmic sperm injection (ICSI) (6) has opened for these couples new chances to have their own genetic offspring. Herein we report the first situation where a patient with idiopathic HH remaining persistently azoospermic after hormonal treatment, in which testicular sperm extraction (TESE) yielded spermatozoa for ICSI.

CASE REPORT

A 30-years-old man was diagnosed 10 years ago to suffer from idiopathic, prepubertal HH. Informed consent for all procedures was obtained. He consulted the endocrinologist because of delayed puberty. He was 1.70-m tall and weighed 78 kg. He did not need to shave his chin or upper lip and had no growth of pubic hair. There were no anosmia or visual field defects and a background of brain trauma was also lacking. No family history of infertility or defective sexual development was recorded. Serum follicle stimulating hormone (FSH) levels were 1.7 mIU/mL, luteinizing hormone (LH) levels were 0.9 mIU/mL, and testosterone was in the female range (0.59 ng/mL). Computerized tomographic scans of the pituitary, hypothalamus, and suprasellar areas did not reveal any abnormal growth. He received testosterone treatment (Testoviron Depot™, 150 mg every 15 days) during 10 years.

He was referred to our center for infertility treatment. Physical examination revealed normal development of secondary sexual characteristics, although a wide hip was evident. Both testes were in the scrotum, but they were soft and small (4 and 5 mL). Epididymes were also small without presenting dilatations. Vasa deferentia were both palpable, although very thin. No

varicoceles were detected. Hormonal profile was as follows: FSH 0.7 mIU/mL, LH 0.45 mIU/mL, testosterone 9.5 ng/mL, and prolactine 9.2 ng/mL. Semen analysis showed azoospermia after centrifuging the whole ejaculate, with reduced volume (1.8 mL), normal pH, and positive fructose test.

Testosterone administration was discontinued and gonadotrophin treatment was initiated with human chorionic gonadotrophin 2500 IU (hCG, Profasi™; Serono) and FSH 150 IU (NeoFertinorm™, Serono) three times a week. Seminal analysis after 3 and 6 months of treatment showed azoospermia. After 9 months of therapy, testicular volume has significantly improved bilaterally (9 and 10 mL), but the patient remained azoospermic. Serum FSH was 2.9 mIU/mL, serum LH <1 mIU/mL, and serum testosterone 7.9 ng/mL. Three months later (12 months after starting treatment) centrifugation of the sperm pellet revealed azoospermia again, and the decision was taken to perform a trial TESE, mainly due to the patients' requirement.

Testicular sperm extraction was carried out by an open left testicular biopsy. Three testicular parenchyma fragments were obtained from different areas of the testes. An additional tissue sample from one of the biopsied areas was sent for histological examination. The tissue fragments were immediately placed on a Petri dish containing Sperm medium (MediCult, Jyllinge, Denmark) and they were dispersed mechanically with the help of two sterile scalpel blades to eventually release the spermatozoa from the seminiferous tubules into the medium, as previously described (7). Motile spermatozoa were observed in the medium and were cryopreserved in pills using Sperm Freezing Medium (MediCult, Yllinsge, Denmark) as previously described (7).

On histological examination an average of 63 tubules per section were assessed. Eight of them were severely hyalinized, while the rest showed spermatogenesis until mature spermatozoa level. An average of 12 mature spermatids per tubule (range 2–25) was assessed. These observations were associated with a moderate basal fibrosis. No Leydig cells hyperplasia was observed. Moderate hypospermatogenesis with focal tubular hyalinization was the histological diagnosis.

Cryopreserved testicular spermatozoa were used for ICSI 2 months later. Multiple follicular development was stimulated with gonadotropins after a downregulation with GnRH antagonists. Nine oocytes were collected by ultrasound-guided transvaginal route and eight of them were

microinjected with motile, frozen-thawed testicular spermatozoa. Four oocytes were normally fertilized and, on day 3, four good-quality embryos developed. Three embryos were transferred to the uterine cavity but, unfortunately no pregnancy was achieved.

DISCUSSION

In a high proportion of patients suffering from HH fertility can be achieved using gonadotropins or pulsatile administration of GnRH, but treatment courses should be continued for 2 or more years. In some trials using gonadotropins, treatment was maintained as long as 42 months (8) or 56 months (9). The reason for prolonging treatment over time is that many HH do not normalize their spermiograms after treatment, but fertility can be achieved even with low sperm concentrations (10). However, maintenance of treatment during so long periods of time has important consequences both from economical and psychological point of view. Also, the patients become anxious about the restoration of spermatogenesis. In the ICSI era, assisted reproductive techniques can anticipate pregnancy in couples with subnormal sperm counts. We also must think that time is against good results, and prolonging male treatments on time makes, conversely, women chances diminish.

In most HH patients, sperm appears in the ejaculate after several months of hormonal treatment. Kliesch *et al.* (11) described a median time of 8.7 months to induce spermatogenesis in patients with isolated HH and 6.7 months in those with hypopituitarism. Spermatogenesis was achieved after a median of 9 months of treatment with gonadotropins in the series analyzed by the European Metrodin HP Study Group (1997) (12) reported a median time of 5 months to obtain a positive sperm count. More recently, Bouloux *et al.* (13) reported a median time to initiation of spermatogenesis of 9 months using recombinant human FSH. In the large series of 76 idiopathic HH men treated with pulsatile GnRH by Pitteloud *et al.* (14) all patients with prior complete or partial pubertal development and half of patients with absent puberty had sperm on their ejaculates by 6 months of therapy. It can be concluded, therefore, that the vast majority of HH patients start spermatogenesis during the first year of treatment, whatever the therapeutic modality used.

Failure to achieve a spermatogenic response is generally attributed to underlying conditions, such as primary testicular failure (e.g. secondary to cryp-

torchidism), hCG desensitization with production of hCG antibodies or growth hormone deficiencies (5,15,16). Testicular biopsy can give some clues about the reason of failure in patients who do respond properly to the standard therapies (12). In our patient there was a disagreement between the increase in testicular volume and the persistent absence of spermatozoa in the ejaculate after 12 months of treatment and therefore we decided to perform a trial TESE. Histological diagnosis was moderate hypospermatogenesis with an average of 12 mature spermatids per tubule, and this may suggest an associated obstruction (not observed neither in the exploration nor the surgery), since the theoretical threshold of 6–7 mature spermatids per tubule needed to detect spermatozoa in the ejaculates was exceeded (17).

In conclusion, the case report here presented clearly demonstrates that the currently available assisted reproduction techniques are of value in patients with HH not responding properly to hormonal treatments.

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