ICSI using testicular sperm in male hypogonadotrophic hypogonadism unresponsive to gonadotrophin therapy

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BACKGROUND: The aim of this study was to assess the use of testicular sperm for ICSI in azoospermic men with hypogonadotrophic hypogonadism unresponsive to gonadotrophin therapy. METHODS: Fifteen patients with hypogonadotrophic hypogonadism who remained azoospermic after hormonal treatment underwent testicular sperm extraction (TESE) and ICSI. These men were recruited from the Egyptian IVF centre over a period of 4 years. All patients were given 75 IU hMG thrice weekly and 5000 IU hCG once or twice weekly for >6 months prior to attempting ICSI/TESE. RESULTS: In 11 out of 15 patients (73%), sperm could be retrieved from testicular tissue and were used for ICSI. Two chemical pregnancies resulted but no clinical pregnancies. Nine patients continued gonadotrophin therapy for another 6 months. Sperm appeared in the ejaculate of three of them. The remaining six patients underwent another ICSI cycle, one using cryopreserved sperm and five underwent a second TESE. One chemical pregnancy and three clinical pregnancies were established. One ongoing, one singleton and one twin pregnancies resulted in the delivery of three healthy babies. In total, of 17 ICSI cycles performed using testicular sperm retrieval, the fertilization rate was 41.7% and the cumulative pregnancy rate was 20%. CONCLUSIONS: The use of testicular sperm for ICSI is a treatment option that can be offered to azoospermic males with hypogonadotrophic hypogonadism either not responding or reluctant to continue hormonal treatment. However, prolonged hormonal treatment may improve TESE/ICSI results.

Key words: azoospermia/hypogonadotrophic hypogonadism/ICSI/testicular sperm extraction

Introduction

The induction of spermatogenesis in men with hypogonadotrophic hypogonadism can be successfully achieved using gonadotrophins or GnRH (Hoffman and Crowley, 1982; Finkel et al., 1985; Schopohl, 1993; Büchter et al., 1998). Generally, therapy is initiated with hCG for 6–12 months to stimulate testosterone secretion before adding hMG. Occasionally hCG alone may result in the appearance of sperm in the ejaculate (Vicari et al., 1992). However, prolonged continuous hormonal replacement therapy is necessary to achieve satisfactory outcomes. The time to first appearance of sperm in the ejaculate varies considerably from patient to patient. At least 3–6 months are needed, but treatment may extend >36 months (Büchter et al., 1998). In spite of low semen quality, pregnancy may occur in a high proportion of cases (Vicari et al., 1992; Liu et al., 1988). However, full spermatogenesis and pregnancy may not be achieved in all cases despite prolonged treatment. Compliance with treatment can be limited due to the cost, inconvenience and discomfort associated with frequent injections over a long time.

Following the first reports of ICSI in humans, it has now become the standard treatment for severe male factor infertility (Mansour, 1998). In addition to the use of ejaculated sperm, azoospermic patients can be treated successfully with ICSI using surgically retrieved sperm from the epididymis or the testis.

The use of new assisted reproductive techniques in combination with medical treatment for hypogonadotrophic hypogonadism is rarely reported in the literature, as most pregnancies occur spontaneously once semen parameters improve. Pregnancies after IVF using sperm from patients with Kallmann’s syndrome have been reported (van de Berk et al., 1991; Tournaye et al., 1992; Smith et al., 1993). Yong et al. (1997) described a case of idiopathic hypogonadotrophic hypogonadism of post-pubertal onset where early intervention with ICSI using ejaculated sperm was attempted following 9 months of gonadotrophin therapy. Although three embryos were transferred, no pregnancy resulted. Medical therapy continued, with a subsequent spontaneous pregnancy after 16 months of treatment. The authors concluded that ICSI procedures should be delayed until final testicular maturation. In another case series including 42 patients, three of the 28 pregnancies (10.7%) resulted from the use of ICSI (Büchter et al., 1998). The authors did not mention the total number of patients who underwent ICSI.
The aim of this study is to report the use of testicular sperm for ICSI in azoospermic males with hypogonadotrophic hypogonadism unresponsive to gonadotrophin therapy.

Materials and methods
The study included 15 infertile azoospermic patients attending the Egyptian IVF–ET Center over the past 4 years with previous diagnosis of hypogonadotrophic hypogonadism. All 15 patients had rather small-sized testes, 6–8 ml in volume with no significant increase in size after hormonal therapy. Their ages ranged from 29 to 52 years (mean 38.71 ± 6.2). The diagnosis of hypogonadotrophic hypogonadism was based on azoospermia, low levels of FSH, LH and testosterone. One patient had classical Kallman syndrome while the rest had isolated hypogonadotrophic hypogonadism. None of the patients had cryptorchidism, or abnormal karyotype. Two patients had diabetes mellitus type II. All patients had previous attempts of treatment with different forms of gonadotrophins over variable periods. They all had history of failed puberty. Variable degrees of virilization were observed in all patients because of the previous hormonal replacement therapy.

Before inclusion in our ICSI programme, all patients received 75 IU hMG thrice weekly and 5000 IU hCG once or twice weekly for ≥6 months. The dose of hCG was adjusted according to the testosterone level. Normal serum testosterone concentration in the adult male lies between 12 and 40 nmol/ml (Behre et al., 2001). Semen analysis was performed every 3 months. Only patients who remained azoospermic after hormonal treatment were offered the treatment option of TESE/ICSI. All patients agreed through a written consent to the procedure. They were informed about all risks of transmitting infertility problems to their offspring.

General, pelvic examination and transvaginal ultrasonography were routinely performed on all female partners and no abnormalities were found. Routine laboratory tests including liver and kidney function tests were performed. Ovulation induction was performed using GnRH analogue protocol. Ovarian stimulation and oocyte retrieval ICSI were performed as described previously (Mansour et al., 1994).

TESE procedure
Open testicular biopsies were performed under local infiltration anaesthesia using a mixture of 1:1 bupivacaine and lidocaine as previously described (Fahmy et al., 1997). A rapid search for sperm was done, and, if no sperm suitable for injection were found, another biopsy was taken either from the same site or from other sites. If still no sperm were found, another biopsy was performed from the other tests. An extra piece of testicular tissue was obtained, fixed in Bouin’s solution and was used later to prepare 4 μm thin paraffin sections stained with haematoxylin and eosin. The collected pieces of testicular tissues were minced in a 200–500 μl droplet of HEPES-buffered Earle’s medium (Medicult, Denmark) in a Petri dish (Falcon, cat. No. 3001; Becton Dickinson, UK) using two G28 needles. Micro-droplets from the suspension of minced testicular tissues were directly transferred to the injection dish and placed carefully in the micro-droplets previously prepared under mineral oil in an injection dish. The micro-droplets containing the suspension of the minced testicular tissue were examined carefully under the inverted phase microscope. If no sperm were found after a rapid search, the rest of the testicular tissue suspension was distributed in the injection dish and examined carefully for 2–3 h by at least two embryologists who searched for sperm. The injected oocytes were checked the next morning for 2-pronuclear formation and the embryo transfer was done on day 2 or 3 after ovum retrieval.

Results
In 11 out of 15 patients (73%), sperm could be retrieved from testicular tissue and were used for ICSI. In five cases, multiple biopsies were needed from both testes and a prolonged search for 2–3 h was performed to collect sufficient sperm to inject all the retrieved oocytes. In five cases, sufficient number of sperm could be retrieved from one testis, but not enough for cryopreservation. In all patients except two, sperm with in situ jerky movements were retrieved and used for injection. In one patient, sperm could be readily retrieved and excess sperm were cryopreserved for future trials. Two chemical pregnancies resulted but no clinical pregnancies. At this stage, six patients dropped out from the study (four with no sperm found and two from the remaining 11 patients).

Nine patients continued gonadotrophin therapy for another 6 months. Sperm appeared in the ejaculate of three of them. The remaining six patients underwent another ICSI cycle, one using cryopreserved sperm and five underwent a second TESE. In all patients, sperm could be retrieved. In two patients, sperm could be readily retrieved without any need for prolonged search compared to a more difficult first TESE with excess sperm cryopreserved for future trials. One chemical pregnancy and three clinical pregnancies were established, resulting in the delivery of three normal offspring one is still ongoing. Two of the three patients with sperm appearing in the ejaculate dropped out from the study. The remaining patient continued on hormonal therapy and clinical pregnancy was achieved following ICSI, resulting in a normal male. Figure 1 illustrates the flow chart of our study and its outcome.

Table I demonstrates the summary results of our study. In total, 17 cycles were performed using testicular sperm retrieved from patients with hypogonadotrophic hypogonadism. The fertilization rate was 41.7%, the clinical pregnancy rate was 17.6% per cycle and the cumulative pregnancy rate was 17.6% per patient. It should be emphasized that the three clinical pregnancies achieved resulted from the repeated six ICSI cycles. No post-operative complications were reported and no clinically detectable decrease of testicular size was noticed up to 6 months after the procedure. The histopathological findings of all patients are illustrated in Table II. It is noteworthy that the basal FSH and testosterone levels of the three patients with pre-pubertal histology were low and were normalized during the treatment period prior to ICSI.

Discussion
Among males, Kallmann syndrome or idiopathic hypogonadotrophic hypogonadism (IHH) has a prevalence of ~1:10 000 (Seminara et al., 1998). As indicated by a positive family history or results of mutation analysis, a primary genetic basis of Kallmann syndrome or IHH is certain in a minority of patients (Meschede and Horst, 1999). The pedigree of patients (Meschede and Horst, 1999). The pedigree of patients
The protocols used for treatment of hypogonadotrophic hypogonadism vary widely. Two thousand international units of hCG may be given up to three times weekly for 3–6 months either alone or with 150 IU hMG (European Metrodin HP Study Group, 1998). Pulsatile GnRH therapy is also used for hypothalamic disorders, but its advantage over gonadotrophins is debated. Some reports show improved results with GnRH and others show no difference (Schopohl, 1993; Liu et al., 1988). This variation may be due to wide differences in the individual response to treatment. Testicular maldescent and small testicular volume are generally considered as negative prognostic factors (Ley and Leonard, 1985). However, other authors suggest that maldescented testes do not necessarily produce poor response to treatment (Saal et al., 1991). Even in patients who initially have a very small testicular volume, successful therapy is possible (Büchter et al., 1998). A positive response to hormonal treatment in patients with hypogonadotrophic hypogonadism may be related to residual function of the pituitary gland that varies individually (Shargil, 1987).

Most studies emphasize the need for long-term treatment in hypogonadotrophic hypogonadism. However, the retrospective nature of much of the work in this area makes assessment of the rate of the non-compliance and the true success rate rather difficult. Those patients discontinuing treatment at early stages are unlikely to be included in retrospective studies, which primarily look at long-term outcomes such as initiation of spermatogenesis and pregnancy. A positive response to hCG/hMG combinations as shown by positive sperm count varies between 40 and 70% (Vicari et al., 1992; Schopohl, 1993; Liu et al., 1988). In a large prospective study carried out on men with hypogonadotrophic hypogonadism, an overall response rate of (80%) was reported (Burgues et al., 1997).

Figure 1. Flow chart of the sequence of events in the study. TESE = testicular sperm extraction.

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<tr>
<th>Table I. Results of testicular sperm extraction/ICSI in 15 patients with hypogonadotrophic hypogonadism</th>
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<td>Successful retrieval</td>
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<td>Fertilization rate (17 cycles)</td>
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<td>Values in parentheses are percentages.</td>
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<th>Table II. Histopathological findings in 15 patients with hypogonadotrophic hypogonadism</th>
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<td>Histopathology</td>
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<tr>
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<td>Arrest at primary spermatocyte stage</td>
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<td>Arrest at spermatid stage</td>
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<td>Hypospermatogenesis</td>
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<td>Arrest with mixed fibrotic changes</td>
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In our study, continuous gonadotrophin therapy was given for >6 months before offering assisted conception. The absence of a significant increase in testicular size and the persistence of azoospermia marked the unresponsiveness to hormonal treatment. This poor response may be related to initial small-sized testes and increased patients’ age. FSH, through its effect on Sertoli cells and blood–testicular barrier, has an important role in the control of testicular microvessularity (Causio et al., 2002). Repeated interrupted cycles of hormonal treatment can, therefore, lead to testicular fibrotic changes. This may deter any expected future effect of hormonal therapy. In the present study, all patients gave history of intermittent hormonal therapy, which in our opinion may have contributed to slow response observed after hormonal treatment. It is of note that gross tubular hyalinization was seen in the testicular histopathology of three patients. The fertilization rate (41.7%) and the pregnancy rate per ICSI cycle (17.6%) are both significantly lower than our previously reported rates in obstructive cases, 57.9 and 34.5% respectively, and very similar to those reported in patients with non-obstructive azoospermia, 41.2 and 16.6% respectively (Fahmy et al., 1997).

Because hormonal treatment for hypogonadotrophic hypogonadism necessitates long periods of drug intake, compliance of the patients is considered an issue of substantial importance. Eight (53%) out of 15 patients dropped out from the study. Two patients dropped out despite the appearance of sperm in their ejaculate following 12 months of hormonal therapy. Although prolonged hormonal treatment in patients with hypogonadotrophic hypogonadism may result in better sperm retrieval rates and perhaps even normal pregnancy (Yong et al., 1997), we think that an early offer of assisted conception to patients with hypogonadotrophic hypogonadism under hormonal treatment at an earlier stage can increase patients’ compliance.

In conclusion, sperm appear in the testes long before their appearance in semen in patients with hypogonadotrophic hypogonadism under HRT. ICSI using testicular sperm can be offered to obtain pregnancy for patients who are either not responding or reluctant to continue hormonal treatment to avoid patient non-compliance. However, prolonged hormonal treatment should be encouraged for the longest possible period to improve TESE/ICSI results.

References

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