High implantation and pregnancy rates with testicular sperm extraction and intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia


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Introduction

High fertilization and pregnancy rates have been reported with testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) with spermatozoa obtained from testicular biopsies in obstructive azoospermic males. In non-obstructive azoospermia the testicles are the only source of sperm cells, and the only possible treatment model is a combination of TESE and ICSI. Hormone levels, testicular volume determinations and testicular biopsy results are not uniform enough to select the candidates for successful TESE. In non-obstructive cases the only way to locate spermatozoa may be to extract multiple tissue samples from different parts of the testicles. The classical belief regarding testicular spermatozoa should be questioned as sufficiently mature spermatozoa for fertilization can be obtained from the testicles. Pregnancies have even been achieved with immature spermatozoa in the early phase of spermatogenesis. Devroey et al. (1995) reported fertilization and pregnancies after TESE and ICSI in non-obstructive azoospermic cases.

Material and methods

In 32 infertile couples with obstructive and non-obstructive azoospermia TESE and ICSI were performed between January and June 1995 in Sevgi Hospital Assisted Reproductive Technologies Unit. Azoospermic males were evaluated by genital examination, hormonal assessment and by ultrasound to determine testicular volume. Histological examinations of testicular biopsy specimens were performed during the diagnostic work-up. In non-obstructive cases a TESE trial was carried out to estimate in which cases spermatozoa were obtainable. The histology of 16 patients for whom the diagnostic TESE was performed, revealed maturation arrest (n = 6), severe hypospermatogenesis (n = 8) and Sertoli cell only (n = 2).

In 16 obstructive azoospermic males with previous biopsy results a scrotal exploration was performed and when no spermatozoa or no motile spermatozoa were found in the epididymis a testicular tissue sample was taken. Cases in which spermatozoa were not obtained were excluded in this study.

Sperm sampling and preparation

Testicular tissue samples were obtained by open biopsy on the same day that the oocytes were retrieved. The tissue sample was taken under local anaesthesia through a 2–3 cm scrotal incision and testicular sperm extraction was performed. The obtained testicular tissue was placed in a Falcon tube (Becton Dickinson, NJ, USA) containing 1 ml of HEPES-buffered Earle’s medium (Gibco, Eggenstein, Germany). The testicular tissue which was progressively divided into small segments, was gently crushed between micro-needles in a Petri dish containing HEPES-buffered medium to obtain a suspension of spermatozoa. The suspension of spermatozoa was then transferred into a Falcon tube and rotated for 50–60 s with a vortex. Isolation of a single spermatozoon was considered satisfactory for ICSI. The
suspension was treated on a mini-column of Percoll gradient (90-70-50%) to reduce the debris and red blood cells. After 10 min centrifugation at 300 g the 70 and 90% Percoll suspensions were isolated and freed from all debris and eventually from red blood cells. The testicular tissue solution was kept in the incubator (5% CO2 in air) at 37°C until the ICSI procedure.

**Ovarian stimulation**

Ovarian stimulation was carried out with the combination of GnRH agonist (GnRHa; Suprefact: Hoechst, Germany) and injection of follicle stimulating hormone (FSH) (Metrodin: Serono, Italy) and human menopausal gonadotrophin (HMG) (Humegen: Organon, Turkey). Ovulation was induced by 10 000 IU HCG (Pregnyl: Organon, Turkey) injection. Oocytes were retrieved 36 h after HCG administration by vaginal ultrasound-guided puncture of the ovarian follicles.

**Oocyte preparation**

The cumulus-corona cells complexes were removed by incubating in a solution of HEPES-buffered Earle’s medium, containing 80 IU/ml hyaluronidase (type VIII, specific activity 320 IU/mg: Sigma, St Louis, MO, USA) for 30 s and by aspiration of the cell complex in and out of a hand-drawn glass pipette. The oocytes were assessed under an inverted microscope at ×200 magnification for nuclear maturity and cytoplasmic evaluation. Oocytes were incubated in EBSS medium at 37°C in an atmosphere of 5% CO2 with air covered by paraffin oil. Only metaphase II oocytes were used for microinjection.

**Intracytoplasmic sperm injection (ICSI) procedure and preparation of pipettes**

These procedures have been described by Van Steirteghem et al. (1995).

**Assessment of fertilization—embryo cleavage and establishment of pregnancy**

The oocytes were observed for the presence of pronuclei 16–18 h after ICSI. Fertilization was assessed as normal when two clearly distinct pronuclei containing nucleoli were present. The state of embryo cleavage and quality were assessed after a further 24 h in vitro culture. The embryos were evaluated according to the blastomere size equality and the relative proportion of anucleate fragments. A maximum of four embryos were transferred in exceptional cases when all the available embryos had >50% fragmentation.

Pregnancy was confirmed by detecting increasing serum HCG concentrations 12 and 16 days after embryo transfer. Clinical pregnancy was diagnosed by ultrasonography at 7 weeks of pregnancy. All couples were counselled and agreed to have prenatal diagnosis. Prenatal diagnosis was performed by amniocentesis at 16 weeks of pregnancy. They were also informed about a prospective follow-up study of the children born after ICSI.

Statistical analysis was performed by χ² and Fisher’s exact test. Significance was defined as P < 0.05.

**Results**

A total of 32 TESE and ICSI cycles were performed, 288 oocytes were obtained and 242 were injected (84%). The number of injected metaphase II oocytes with two pronuclei (2PN) was 123 (50.8%).

In the obstructive azoospermia group the fertilization and cleavage rates were 65.3% and 62.3%, respectively. A total of 44 oocytes were transferred in 16 cycles. Ten pregnancies were achieved in total (62.5%) and three pregnancies resulted in clinical abortion (30%). The ongoing pregnancy rate in the obstructive azoospermia group per embryo transfer was 43.7 and a high implantation rate was achieved (30%). Three sets of triplets, one set of twins and six singleton pregnancies were achieved. Three singleton pregnancies resulted in abortion.

In the non-obstructive azoospermic group the fertilization rate with 2 PN was 34%. Four cases resulted in total fertilization failure (25%). The number of unfertilized intact oocytes was significantly higher (48.2%) in this group compared to the obstructive patients group (P < 0.001). A high cleavage rate was achieved (81.5%). Five pregnancies were obtained (41.6%), one of which resulted in clinical abortion (20%). The implantation rate was 26.6%; two sets of twins and three singleton pregnancies were achieved. One singleton pregnancy resulted in clinical abortion.

Triple pregnancies were reduced successfully to twins by embryo reduction procedure. To date, four sets of twins and one singleton pregnancy have resulted in live healthy births.

It was observed that sperm motility and density was better in the obstructive group. After a 2 h incubation period, spermatozoa became more motile but never progressive. In

| Table I. Fertilization and cleavage rates after testicular sperm extraction and intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia. |
|---------------------------------|-----------------|-----------------|
| Non-obstructive | Obstructive | Total |
| No. of cycles | 16 | 16 | 32 |
| No. of oocytes retrieved | 145 (50.3) | 143 (49.6) | 288 |
| No. of oocytes | 112 (77.2) | 130 (90) | 242 (84.0) |
| (metaphase II) injected | 38 (33.9) | 85 (65.3) | 123 (50.8) |
| No. of oocytes fertilized (with 2PN) | 54 (48.2) | 24 (18.4) | 78 (32.2) |
| No. of intact unfertilized oocytes | 31 (81.5) | 53 (62.3) | 84 (68.2) |
| No. of embryos cleaved | 30 (40.5) | 44 (59.4) | 74 |

The values in parentheses are percentages.

P < 0.01.

P < 0.05.

2PN = two pronuclear.

| Table II. Clinical and ongoing pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive and obstructive azoospermia. |
|---------------------------------|-----------------|-----------------|
| Non-obstructive | Obstructive | Total |
| No. of started cycles | 16 | 32 | 48 |
| No. of ETs | 12 (42.8) | 16 (57.1) | 28 |
| No. of clinical pregnancies (PR/ET) | 5 (41.6) | 10 (62.5) | 15 (53.5) |
| No. of clinical abortions | 1 (20.0) | 3 (30.0) | 4 (26.6) |
| No. of ongoing pregnancies (PR/ET) | 4 (33.3) | 7 (43.7) | 11 (39.2) |
| No. of ongoing pregnancies/started cycles | 4 (25) | 7 (43.7) | 11 (34.3) |
| Implantation rate (%) | 26.6 | 30 |

The values in parentheses are percentages.

P < 0.01.

P < 0.05.

ET = embryo transfer; PR = pregnancy rate.

There were three sets of triplets and three sets of twins.
the non-obstructive group the spermatozoa were not as mature as those in the obstructive group, due to the spermatogenic defect, and they were mostly immobile or sluggishly motile after the same incubation periods. The ICSI procedure took longer with these immature spermatozoa as they stuck to the inside and to the tip of the pipette.

Discussion

A large number of patients with severe oligoasthenoteratozoospermia can benefit from the application of ICSI. Van Steirteghem et al. (1993a,b) reported high fertilization and pregnancy rates with ICSI despite extremely poor semen parameters.

It has been proven that for the males who have obstructive azoospermia with congenital absence of the vas deferens or irreparable obstructive azoospermia the combination of microsurgical epididymal aspiration (MESA) and ICSI yields high fertilization and pregnancy rates comparable to those obtained with ejaculated spermatozoa (Tournaye et al., 1994). In the case of totally destroyed epididymis or non-obstructive azoospermia with severe spermatogenic defect the testicles are the only source of sperm cells. Testicular spermatozoa can be used for ICSI and yield high fertilization and pregnancy rates (Devroey et al., 1995). Silber et al. (1995) reported high fertilization and pregnancy rates after ICSI with spermatozoa from the testicular tissue.

In this study, obstructive azoospermic males in whom PESA (Craft, 1995b) and MESA were impossible due to totally destroyed epididymis where the testicles were the only source of sperm cells and non-obstructive azoospermic males with severe spermatogenic defect were included. More recently, testicular sperm aspiration (TESA) have been described as an alternative method by Craft and Tsirigotis (1995).

These simplified sperm recovery methods may be more likely to yield spermatozoa in patients with focal spermatogenesis. If multiple sites are sampled, an open biopsy might not include an active focal area. A 21-33 gauge butterfly needle is passed directly into the testis through the scrotal skin under local sedation or general anaesthesia using a 10 ml attached syringe to create a strong negative pressure. Multiple biopsies can be carried out making an open biopsy unnecessary (Craft et al., 1995; Tsirigotis et al., 1995).

The FR was higher in the obstructive azoospermic group, probably due to normal spermatogenic activity in their testicular biopsies. Thus a lower fertilization rate and a higher number of unfertilized oocytes were obtained in the non-obstructive group (P < 0.01).

The FR was 85 and 33.9% respectively in the obstructive and non-obstructive groups. In the latter group this rate may be due to using less mature spermatozoa, but the maturity could not be confirmed without a Hoffman modulator contrast system.

Although severe spermatogenic defects with Sertoli cell only, severe hypospermatogenesis, tubular hyalinization or bilateral maturation arrest can cause immature sperm cells, Devroey et al. (1994) suggested that testicular spermatozoa had gained full maturity. They were also surprised to observe that the fertilization and cleavage rates were remarkably similar in patients with severe spermatogenetic defects to those they observed using testicular spermatozoa from men with normal spermatogenesis in cases of obstructive azoospermia (Devroey et al., 1994; Silber et al., 1994). However, in our study the FR was significantly higher in the obstructive group (P < 0.01). The fertilization rate may be affected by the degree of spermatogenetic defect in the non-obstructive group.

In four cases a total fertilization failure was observed in the non-obstructive group. This was possibly due to using the spermatozoa either in the early phase of the spermatogenesis or with a very severe spermatogenetic defect (one case with Sertoli cell only and three with maturation arrest). Fewer spermatozoa than oocytes were obtained in the Sertoli cell only case.

A total of 74 embryos were transferred. The embryo quality was similar in both groups. The connection between embryo quality and sperm origin needs to be studied further. In both groups a high implantation rate was obtained. The implantation rate was 30% in the obstructive and 26.6% in the non-obstructive group by yielding high multifetal pregnancies. Three sets of triplets, three sets of twins and five singleton pregnancies were obtained.

In spite of similar implantation rates, a statistically significant difference was observed between the clinical and ongoing pregnancy rates in both groups (P < 0.01, P < 0.05 respectively). In the obstructive azoospermia group the higher fertilization and ongoing pregnancy rates may be due to normal spermatogenesis and the use of more mature spermatozoa. It is also thought that higher on-going pregnancy rates were obtained in the obstructive group due to a higher number of transferable embryos.

Although the number of patients was low, these optimistic results of high implantation and pregnancy rates with testicular spermatozoa in combination with ICSI provide hope in the treatment of males with azoospermia.

References

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