Patients with absolutely immotile spermatozoa and intracytoplasmic sperm injection


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The microinjection of completely immotile spermatozoa may impair the outcome of intracytoplasmic sperm injection (ICSI). Eleven couples underwent an initial ICSI cycle with 100% immotile freshly ejaculated spermatozoa. Two-pronuclear fertilization ensued in 18 of 145 (12.4%) successfully injected oocytes. None of these cycles resulted in a pregnancy. Nine couples underwent ICSI in subsequent cycles (n = 16). Ejaculated spermatozoa were injected in 15 cycles and testicular spermatozoa in one cycle. In 10 of the 15 cycles, motile spermatozoa were available at the time of injection. Motile testicular spermatozoa could also be injected. In the subsequent cycles, 91 of 176 (51.7%) successfully injected oocytes fertilized normally and four patients became pregnant. In the subsequent cycles where again immotile spermatozoa had to be injected no pregnancies occurred. In four subsequent cycles embryo cryopreservation was carried out. After replacement of two frozen-thawed embryos one additional pregnancy was obtained. In all, five healthy infants were born. It has been ascertained that motile spermatozoa can be detected either in repeated ejaculates or after testicular biopsy. The causes of total asthenozoospermia are variable and the problem is a sporadic rather than a permanent condition.

Key words: immotile spermatozoa/intracytoplasmic sperm injection/pregnancy/two-pronuclear fertilization

Introduction

Intracytoplasmic sperm injection (ICSI) with freshly ejaculated spermatozoa gives high fertilization and pregnancy rates in couples with extreme male factor infertility (Palermo et al., 1993; Van Steirteghem et al., 1993a,b). Whereas sperm concentration and sperm morphology of the ejaculated samples seem not to interfere with ICSI results, the injection of a totally immotile spermatozoon may have a negative effect on fertilization and pregnancy rates (Liu et al., 1995; Nagy et al., 1995). In particular, the injection of immotile spermatozoa from necrozoospermic samples, in which all spermatozoa are dead, may result in a poor outcome (Tournaye et al., 1996).

This study was undertaken to see if couples with only totally immotile spermatozoa in an initial ICSI cycle could benefit from subsequent ICSI cycles. We aimed to investigate whether complete asthenozoospermia is a permanent or sporadic phenomenon and whether the fertility prognosis in these couples for whom only immotile spermatozoa are available in a first treatment cycle is also impaired in subsequent cycles.

Materials and methods

Patients

A cohort of 11 couples was selected. These couples, previously described by Nagy et al. (1995), underwent ICSI with 100% immotile spermatozoa between October 1992 and November 1993. The female patients were stimulated by a desensitizing protocol of gonadotrophin-releasing hormone (GnRH) analogues (buserelin; Suprefact, Hoechst, Brussels, Belgium) combined with human menopausal gonadotrophins (HMG, Humegon from Organon, Oss, The Netherlands or Pergonal from Serono, Brussels, Belgium). Vaginal ultrasound-guided ovum retrieval was scheduled 36 h after the administration of human chorionic gonadotrophin (HCG; Pregnyl from Organon or Profasi from Serono). This stimulation regimen has been described in detail previously (Smits et al., 1988). Cleaving embryos were transferred 48 h after oocyte retrieval. The luteal phase was supported by micronized progesterone (600 mg) administered intravaginally in three doses (Utrogestan; Piete, Brussels, Belgium) (Smits et al., 1992).

Semen preparation and intracytoplasmic sperm injection

Ejaculated spermatozoa were obtained by masturbation. The patients were asked to respect an ejaculatory abstinence period of 3–5 days prior to the day of oocyte retrieval. Sperm parameters were assessed according to the World Health Organization criteria (WHO, 1992). Whenever enough motile spermatozoa were observed in the preliminary samples, a mixed agglutination reaction (MAR) test was performed. Where all spermatozoa were immotile, at least two preliminary samples were assessed. Only if a sufficient number of immotile spermatozoa were found in the preliminary samples was a vitality test with eosine-Y stain carried out.

The preparation of the spermatozoa for ICSI was as follows: removal of seminal fluid by washing in Earle’s medium and centrifugation at 1800 g for 5 min, two-layer Percoll (95.0–47.5%) centrifugation at 300 g for 20 min and a final concentration step by centrifugation at 1800 g for 5 min (Liu et al., 1994). Immotile spermatozoa were treated in the same way as motile spermatozoa, including the immobilizing step, by mechanically crushing the sperm tail.

The retrieval of spermatozoa from the testicle by excisional biopsy (TESE) and their treatment before the injection procedure have been described by Devroey et al. (1995).

The preparation of the holding and injection pipettes, as well as the injection procedure itself, have been described in great detail previously by Van Steirteghem et al. (1995).

Assessment of fertilization and embryonic development

The oocytes were evaluated for survival and fertilization ~18 h after the injection procedure, by means of an inverted microscope (×200,
Eleven embryos were transferred in five couples and two-pronuclear fertilization was observed in 18 successfully injected oocytes (12.4%), while 12 2PN oocytes started to cleave. Ten out of 18 embryos contained a metaphase-II oocyte, 145 (89.5%) of which were successfully injected with immotile spermatozoa. The injection procedure was successful in 16 out of 18 subsequent cycles. With the exception of one cycle, all spermatozoa were retrieved in at least two treated semen samples. On two occasions all spermatozoa retained the eosin-Y (necrozoospermia), while on three other occasions vitality was 2, 12 and 34% respectively. Sperm vitality was not tested in six samples. Supernumerary embryos with <20% fragmentation were cryopreserved (Van Steirteghem et al., 1994). Only embryos with <50% anucleate fragments were considered suitable for transfer 48 h after retrieval. Supernumerary embryos with <20% fragmentation were cryopreserved (Van Steirteghem et al., 1994).

Establishment and follow-up of pregnancy
Two measurements of increasing serum HCG at least 10 days after embryo transfer confirmed implantation, while clinical pregnancy was confirmed by the visualization of a gestational sac on vaginal ultrasound 5 weeks after embryo transfer.

Results

Initial ICSI cycles
Eleven couples underwent their first ICSI cycle with 100% immotile spermatozoa. The mean ± SD ages of these couples were 31.1 ± 3.9 years for female partners and 32.8 ± 4.2 years for male partners. Sperm characteristics and the detailed outcome of these initial cycles are given in Table I. All oocytes were injected with totally immotile spermatozoa because after an intensive search for several hours no motile spermatozoa were found in at least two treated semen samples. On two occasions all spermatozoa retained the eosin-Y (necrozoospermia), while on three other occasions vitality was 2, 12 and 34% respectively. Sperm vitality was not tested in six preliminary samples because of extreme oligozoospermia. The associated features and possible aetiological factors of the impaired motility of the spermatozoa are given in Table II.

In all, 192 complexes were retrieved. A total of 162 (84.4%) contained a metaphase-II oocyte, 145 (89.5%) of which were successfully injected with immotile spermatozoa. Two-pronuclear fertilization was observed in 18 successfully injected oocytes (12.4%), while 12 2PN oocytes started to cleave. Eleven embryos were transferred in five couples and no embryos were available for cryopreservation. None of the women became pregnant.

Subsequent ICSI cycles
One couple (Table I, couple A) decided to abandon any further treatment while another couple (Table I, couple H) opted for insemination with donor spermatozoa. Nine couples underwent 16 subsequent ICSI cycles with the husband's spermatozoa. In one patient with necrozoospermia (Table I, couple G), spermatozoa were retrieved from the testicle after four unsuccessful fertilization attempts with ejaculated spermatozoa. Table III shows the semen characteristics and outcome of the subsequent ICSI cycles.

Progressive motility was observed in four subsequent cycles, while in three other cycles grade 3 motility was observed. After sperm preparation and an intensive search, some motile spermatozoa were available in four more cycles, i.e. three cycles with ejaculated spermatozoa and one cycle with testicular spermatozoa. Finally, motile spermatozoa were injected in 12 out of 16 subsequent cycles. With the exception of one couple, all had at least one subsequent cycle in which motile spermatozoa were found.

In these 16 subsequent cycles, 240 cumulus–corona–oocyte complexes were retrieved, 202 (84.2%) of which contained a metaphase-II oocyte. The injection procedure was successful.

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**Table I.** Semen characteristics and results of intracytoplasmic sperm injection in the initial cycles

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sperm concentration (&lt;10⁹/ml)</th>
<th>Total motility (%)</th>
<th>Vitality (%)</th>
<th>Motile sperm at injection</th>
<th>No. of MII oocytes</th>
<th>Successfully injected oocytes</th>
<th>No. of 2PN oocytes</th>
<th>No. of embryos transferred</th>
<th>Pregnancy achieved</th>
</tr>
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<td>–</td>
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<td>2</td>
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<td>no</td>
</tr>
<tr>
<td>B</td>
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<td>0 NA</td>
<td>–</td>
<td>–</td>
<td>20</td>
<td>17</td>
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</tr>
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</table>

**Table II.** Associated features and possible aetiologies of ‘absolute’ asthenozoospermia in the initial cycles and the presence or absence of motile spermatozoa in the subsequent cycles. Pregnancies only occurred in the subsequent cycles when motile spermatozoa were injected

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>No. of patients</th>
<th>Motile spermatozoa at injection in subsequent cycles</th>
<th>Pregnancies in subsequent cycles</th>
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<tbody>
<tr>
<td>Leukospermia</td>
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<td>+</td>
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<tr>
<td>Necrozoospermia</td>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Post orchitis</td>
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injected, resulting in a fertilization rate of 65.3%. In 12 subsequent cycles motile spermatozoa were injected, and nine embryos were cryopreserved. A fertilization rate of 34.4% was observed when totally immotile spermatozoa were injected. In 12 subsequent cycles motile spermatozoa were injected, and nine embryos were cryopreserved. A fertilization rate of 51.7%.

Discussion

Sperm motility is enhanced during epididymal maturation and is important for transit from the vagina to the Fallopian tube, penetration of the zona pellucida and processes involved in fertilization. Although total asthenozoospermia is reported at a frequency of one in 5000 men attending an infertility clinic (Eliasson et al., 1977), it is an important problem in andrological infertility since it implies a poor prognosis. A clear relationship between natural conception rates and sperm motility has been observed (Beauchamp et al., 1984). Fertilization rates after regular in-vitro fertilization are disappointing in cases of asthenozoospermia (Mahadevan et al., 1984). The introduction of ICSI, however, opened a new area in the field of assisted reproduction (Palermo et al., 1992, 1993; Van Steirteghem et al., 1993a,b) since it became possible to treat couples suffering from severe oligoasthenoteratozoospermia.

High fertilization and pregnancy rates after ICSI treatment with ejaculated spermatozoa are not related to sperm density and morphology in the semen sample on the day of oocyte retrieval (Nagy et al., 1995; Oehninger et al., 1995). Total sperm motility (progressive and non-progressive) does not affect ICSI results, except in cases where only immotile spermatozoa are available for injection (Liu et al., 1995; Nagy et al., 1995). Two subgroups of patients can be distinguished within the group with complete asthenozoospermia. A first subgroup has immotile spermatozoa in their preliminary samples, but after centrifugation and selection on a Percoll gradient some motile spermatozoa (mostly sluggish) may be observed in the treated sample. We refer to this group as 'virtual asthenozoospermic'. In the 'absolute asthenozoospermic' subgroup, even after sperm treatment, incubation and intensive search, all spermatozoa remain 100% immotile.

All the initial cycles mentioned in this paper have been described previously by Nagy et al. (1995). They observed a significant difference in 2PN fertilization rates in 12 ICSI cycles with 'absolute asthenozoospermic' spermatozoa (10.9%) and 54 cycles with 'virtual asthenozoospermic' spermatozoa (60.2%). In the former, no pregnancies ensued, while in the latter six ongoing pregnancies were observed. Nagy et al. (1995) correlated the fertilization rate with the percentage of viable spermatozoa in the semen samples. Although Goto et al. (1990) successfully injected bovine oocytes with killed spermatozoa and Gearon et al. (1995) obtained a 2PN fertilization rate of 38% with destroyed spermatozoa in humans, there is no evidence that apoptotic or senescent spermatozoa keep their fertilizing capacity in humans. When a dead and consequently immotile spermatozoon is injected into an oocyte, the intact sperm cell can still be seen in the ooplasm 20 h after the injection (Liu et al., 1995).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cycle no.</th>
<th>Sperm concentration ((\times 10^6/ml))</th>
<th>Total motility (%)</th>
<th>Progressive motility (%)</th>
<th>Motile sperm at injection</th>
<th>No. of oocytes injected</th>
<th>Successfully fertilized</th>
<th>No. of 2PN oocytes</th>
<th>No. of embryos transferred</th>
<th>Pregnancy achieved</th>
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\(\text{a}\)Sperm characteristics.  
\(\text{b}\)Patient C became pregnant and delivered after replacement of two frozen-thawed embryos.  
\(\text{c}\)ICSI was carried out with motile testicular spermatozoa.  
\(\text{d}\)Fertilization rate of 51.7%.

Table III. Semen characteristics and results of intracytoplasmic sperm injection in the subsequent cycles.
In order to assess sperm viability, the eosine-Y-stain, based on sperm membrane integrity, is used. Unfortunately, in our series, vitality could not be tested in six couples because of extreme oligozoospermia. Furthermore, this test only supplies a rough estimation of the numbers of living spermatozoa in preliminary samples. Immotile and viable spermatozoa suitable for injection cannot be chosen on the basis of this test because of the dye’s unknown toxic effect on the DNA content of the spermatozoon. We consider the eosine-Y test applicable only for diagnostic purposes.

For future clinical purposes another vitality test has become available. The hypo-osmotic swelling test (HOST), based on the structural and functional integrity of the sperm membrane, was first described by Jeyendran et al. in 1984. Casper et al. (1996) were the first to use the HOST to select viable but immotile spermatozoa for ICSI. They obtained a fertilization rate of 43% in eight ICSI cycles. Although the HOST can be applied successfully to select viable spermatozoa for ICSI, it was not used in our series because its toxicity had yet to be ruled out completely.

Several conditions are known to impair sperm motility. Anti-sperm antibodies, infection of the genital tract (Escherichia coli), necrozoospermia and prolonged periods of anejaculation are among the most common causes. Ultrastructural sperm defects and metabolic sperm defects are less common causes of asthenozoospermia. Frequently, the aetiology of the asthenozoospermia remains idiopathic.

The constant or occasional character of 'absolute' asthenozoospermia in our 11 patients cannot be explained in terms of prolonged periods of anejaculation. Couples were asked to abstain from sexual intercourse for 3-5 days prior to ovum retrieval. In addition, if the first semen analysis revealed only immotile spermatozoa, a second semen sample was requested, so that senescence of the spermatozoa due to a long period of sexual abstinence was not a cause of the asthenozoospermia. None of the tested patients was positive for anti-sperm antibodies.

In our series, two patients had manifest necrozoospermia. The spouse of the patient (Table I, patient G) suffering from chronic prostatitis became pregnant after the use of motile and consequently viable testicular spermatozoa after five previous failed ICSI cycles with ejaculated spermatozoa. In two cycles motile spermatozoa were injected. Tournaye et al. (1996) demonstrated that viable testicular spermatozoa are capable of fertilizing in cases of persistent or occasional necrozoospermia. They recommend performing ICSI in combination with TESE in patients with proven necrozoospermia. The second patient (Table I, patient K), in whom we could not identify a clear aetiology of the necrozoospermia, had motile ejaculated spermatozoa in a subsequent cycle. Unfortunately only one oocyte out of three became fertilized, and no pregnancy resulted. These two cases demonstrate that 'absolute' asthenozoospermia in necrozoospermic patients is not a permanent condition. A contemporary relief of a genital tract obstruction, as in cases of chronic prostatitis, may result in the ejaculation of non-senescent, motile and viable spermatozoa.

Ultrastructural sperm defects were observed in two patients. The injection of immotile spermatozoa from the patient (Table I, patient H) with an axonemal 9+0 defect did not result in any fertilization. The fertilization rate in patient J (Table I), who was suffering from Kartagener's syndrome, was 6 and 10% in the initial and subsequent cycles respectively. In both cycles, only immotile spermatozoa were injected. Beside the low vitality rates in both patients, an explanation for these low fertilization rates is not obvious. Fertilization rates of between 26 and 60% have been reported with subzonal insemination (SUI) in patients with ultrastructural axonemal defects (Bongso et al., 1989; Terriou et al., 1993). Theoretically, the chance of injecting at least one mature and viable spermatozoon subzonoally is considerable higher than in ICSI, since many spermatozoa are injected in SUI (Nijs et al., 1996). If this hypothesis is true, then SUI should be preferred above ICSI in all cases of total asthenozoospermia. Since the superior quality of ICSI compared to SUIZI has already been proven in cases of oligoasthenoteratozoospermia (Van Steirteghem et al., 1993a), we assume that injection of a great number of immotile spermatozoa into the perivitelline space does not result in higher fertilization and pregnancy rates, though this has not been proven in prospective controlled trials. The use of testicular biopsy to find motile spermatozoa in cases of ultrastructural defects has yet to be properly assessed. Ultrastructural aberrations are the result of a spermiogenesis defect and not a spermatogenesis defect (Zamboni, 1987; Baccetti et al., 1993). We can expect that the disorder of the motor apparatus will also be present in the spermatozoa at the testicular level, so that we assume that combination of ICSI with TESE is not primarily indicated in these patients. Recently, however, Kahraman et al. (1997) reported the first birth of a healthy child after an ICSI treatment with immotile testicular spermatozoa in a patient with absolute asthenozoospermia. Besides a varicocelectomy, no other medical problems with a direct negative effect on sperm motility were present.

In seven patients (Table I, patients A–F) extreme oligozoospermia was associated with total asthenozoospermia. Unfortunately, the viability of the spermatozoa of these seven patients could not be tested, since sperm concentration was far too low to carry out the eosine-Y test. Couples A and D had a seminal leukocyte count of $5.3 \times 10^6$ and $1.68 \times 10^6$ respectively, so that, in these cases, asthenozoospermia may be explained in terms of leukospermia. We were astonished by the considerable difference in sperm density in the initial and subsequent cycles from couple F. All patients with extreme oligozoospermia who underwent subsequent cycles had at least one cycle in which motile spermatozoa were injected. The question can be raised as to whether or not defective spermiogenesis is associated with an intrinsic sperm defect leading to motility disorders and poor fertilization.

There is also probably an intra-observer and inter-observer variation, because the search for the few motile spermatozoa present in an apparently completely asthenozoospermic sample is time-consuming, difficult and can lead to misinterpretation. When the outcomes of the initial and subsequent cycles are compared, we can only posit an amelioration in sperm motility as an explanation for the increase in fertilization and pregnancy rates. The low fertilization rates in the subsequent cycles in which immotile spermatozoa were injected confirm the previ-
ous observation that fertilization rates after ICSI are only dependent on the injection of a motile or immotile spermatozoon (Nagy et al., 1995). The present small series demonstrates conclusively that ‘absolute’ asthenozoospermia can be observed in a heterogeneous group and that it is a sporadic rather than a permanent condition, except in cases of ultrastructural sperm defects. Thus, if the injection of ejaculated immotile spermatozoon in an initial cycle leads to poor results, performance of subsequent ICSI cycles with ejaculated spermatozoon is justified. It has been found that motile spermatozoon can be detected either in repetitive ejaculates or after testicular biopsy. Although ICSI has to be performed preferentially with testicular spermatozoon in cases of necrozoospermia, motile, and consequently viable, ejaculated spermatozoon may be observed in subsequent cycles. The use of donor spermatozoon is only indicated if, after repetitive semen analysis, all spermatozoon are immotile, or if abnormal spermatogenesis is confirmed at testicular biopsy or in cases of ultrastructural sperm defects.

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Immotile spermatozoon and ICSI