Different fertilization rates between immotile testicular spermatozoa and immotile ejaculated spermatozoa for ICSI in men with Kartagener’s syndrome: case reports

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We report two cases of infertility treatment in couples where males suffered from Kartagener’s syndrome (KS) and a total absence of motile sperm in the ejaculate. A total of three ICSI cycles was carried out. In all cycles, viable ejaculated or testicular spermatozoa were selected using the hypo-osmotic swelling (HOS) test. Case 1: In the first ICSI cycle total fertilization failure occurred after using ejaculated spermatozoa. In the following cycle testicular spermatozoa were used for ICSI, resulting in 75% fertilized oocytes and a pregnancy. Case 2: In the same ICSI cycle 50% of the oocytes were injected with ejaculated and 50% with testicular spermatozoa. The fertilization rates were 44 and 56% respectively and high quality embryos were achieved in both groups. One single embryo derived from testicular sperm was transferred with a resulting singleton pregnancy. In conclusion, testicular sperm for ICSI seem to have reliable fertilization capacity in men with KS, while ejaculated sperm, even if tested viable, seem more unpredictable. HOS test for selection of viable sperm for ICSI is recommended when ejaculated as well as testicular sperm are used for ICSI.

Key words: hypo-osmotic swelling test/ICSI/immotile cilia syndrome/Kartagener’s syndrome/testicular sperm aspiration

Introduction

The immotile cilia syndrome (ICS) is an autosomal recessive disease characterized by defective ciliary ultrastructure in ciliated cells and affects ~1 in 20 000 newborns. The dynein arms connecting the microtubules are shortened or absent with the consequence of sperm immobility and ciliary epithelial dysfunction (Palmblad et al., 1984). Men with ICS and simultaneous presence of situs inversus and bronchiectasis are referred to as having Kartagener’s syndrome (KS) (Afzelius, 1985).

Total asthenozoospermia (absence of any motile sperm in the ejaculate) is a severe problem. Even when the most advanced assisted reproductive technique such as ICSI are used, fertilization rates have been low using immotile ejaculated sperm (Nijs et al., 1996; Nagy et al., 1998). Even if fertilization is achieved, embryos of lower quality tend to be produced (Nijs et al., 1996) and very low numbers of ongoing pregnancies have so far been achieved using immotile spermatozoa from the ejaculate (Kahraman et al., 1996; Nijs et al., 1996). Several studies have recently reported improved fertilization and ongoing pregnancy rates when using testicular sperm for ICSI compared with ejaculated sperm in cases of total asthenozoospermia (Kahraman et al., 1996; Nijs et al., 1996; Shulman et al., 1999).

To date, only a few reports have been published where fertilization and pregnancies have been successful in couples with male ICS/KS and totally immotile spermatozoa. Total fertilization failure (Nijs et al., 1996; Abu-Musa et al., 1999; Cayan et al., 2001), reduced fertilization rates (Papadimas et al., 1997) but also normal fertilization rates, pregnancies and births (von Zumbusch et al., 1998) have been reported after ICSI with ejaculated immotile spermatozoa. Cayan et al. (2001) reported normal fertilization rates and also a pregnancy in two cases where testicular immotile spermatozoa were used for ICSI.

We report here two cases, where immotile spermatozoa from the ejaculate as well as from testes, have been used for ICSI in couples with male KS. This is the first report where male gametes from both ejaculated and testicular origins have been used for ICSI in the same cycle.

Materials and methods

Case 1

The patient was a 41-year-old male diagnosed with KS on the basis of medical issues including recurrent middle ear infections, sinusitis, bronchiectasis and situs inversus. His semen analysis had repeatedly shown totally immotile spermatozoa but also severe oligozoospermia where only occasional immotile spermatozoa had been present in the ejaculate.

Transmission electron microscopy reveals the severity of defects in sperm tail ultrastructure and therefore is of benefit. However, this was not carried out in this case due to the low number of ejaculated sperm.

Serum FSH, LH and testosterone were normal and the patient also had a normal 46,XY karyotype. Screening of the Y-chromosome did not reveal a deletion, but a rearrangement was found in 25% of spermatozoa.
Therapeutic approach and results

First cycle (November 2001)

After an ovarian stimulation by a long protocol using GnRH agonist (Synarel®; Rydalmere, NSW, Australia) in association with recombinant FSH (Gonal-F®; Serono Australia Pty Ltd, Frenchs Forest, NSW, Australia) 11 oocytes were recovered. Semen analysis confirmed total asthenozoospermia and the hypo-osmotic swelling (HOS) test (World Health Organization, 1999) was used to identify viable sperm for ICSI. In-house sperm washing media was diluted with an equal volume of ‘milli Q’ water (18 ohm) to use in identifying HOS positive spermatozoa. In the ICSI dish a long drop of HOS media was made alongside the polyvinyl pyrrolidone (PVP) and oocyte drops. A small aliquot of washed spermatozoa was placed in the hyper-osmotic long drop and the ICSI procedure started immediately. Those spermatozoa that exhibited head swelling and discernable tail coiling were deemed viable. They were then aspirated with the injection pipette and placed into the 10% PVP solution where they were left for several minutes until the sperm head reduced in size to a normal appearance. The spermatozoa were then stroked across the mid-piece with the injection pipette as if they were motile and injected into mature oocytes.

Injected oocytes were then placed into group culture and checked for fertilization 18 h post-injection.

The ICSI procedure was performed according to the method described by Payne et al. (1995).

Seven of the oocytes were injected with HOS positive sperm. No fertilization occurred.

Second cycle (April 2002)

After using a similar ovarian stimulation protocol as in the first cycle, eight oocytes were recovered. This time testicular spermatozoa recovered by testicular sperm aspiration (TESA) were chosen for ICSI. The testicular sperm retrieval was performed on the day of oocyte retrieval under local anaesthesia. The surgical technique for TESA was performed as described by Westlander et al. (1999). Four needle aspirations were taken from each testis. Testicular tissue was obtained and only immotile spermatozoa were found in culture from both sides. The HOS test was applied to identify viable immotile spermatozoa. All eight oocytes were micro-injected by HOS positive immotile spermatozoa and six oocytes were fertilized. Three days after oocyte retrieval, two good quality embryos were transferred. Three of the remaining four embryos were cryopreserved

A pregnancy test performed 17 days after embryo transfer was positive and a pregnancy scan in the 8th week of gestation revealed a viable singleton pregnancy.

Case 2

The patient was a 33-year-old male whose 28-year-old female partner had not been pregnant in the past. The couple had been married for 3 years. The male had earlier been diagnosed with KS. He had one brother and one sister, both unaffected. His semen analysis had earlier shown a normal sperm count and morphology but totally immotile spermatozoa. The HOS test conducted on immotile sperm from the ejaculate revealed 63% vitality. TEM showed all axonemes with total absence of dynein arms. Serum FSH, LH and testosterone were normal and so was the male karyotype (46,XY).

The couple were interested in the IVF/ICSI programme and agreed to use two different origins of sperm for ICSI for fertilization comparison. Before treatment the couple underwent genetic counseling for ICS/KS. Institutional review board approval was not required to proceed with therapy.

Therapeutic approach and results (September 2002)

After using a similar ovarian stimulation protocol to Case 1, 18 mature oocytes were recovered. The same morning a sperm sample was produced for semen analyses and subsequently TESA from both testes was performed under local anaesthesia. Nine oocytes were microinjected with immotile sperm from the ejaculate and nine with immotile testicular sperm. All sperm microinjected were HOS test positive. Four oocytes were fertilized with ejaculated sperm and five with testicular sperm. One excellent quality embryo derived from testicular sperm was transferred on day 3. Six of the remaining embryos fulfilled the criteria for freezing and were cryopreserved.

A pregnancy test performed 17 days after embryo transfer was positive and a pregnancy scan in the 8th week of gestation revealed a viable singleton pregnancy.

Discussion

Even with the improvement of microinsemination techniques such as ICSI, total asthenozoospermia remains a strongly negative influence on the result of ICSI (Nagy et al., 1995; Kahraman et al., 1996) However, the usage of immotile testicular sperm for ICSI in these cases has dramatically improved fertilization and pregnancy results (Kahraman et al., 1996; Nijs et al., 1996; Shulman et al., 1999). In those patients with ICS and KS the sperm immotility is related to defects in the dynein arms present in the sperm tails. Only a few case reports have so far been published and fertilization and pregnancy results in this patient group have been varying and unpredictable when using immotile ejaculated spermatozoa for ICSI (Papadimas et al., 1997; von Zumbusch et al., 1998; Abu-Musa et al., 1999; Cayan et al., 2001). Usage of testicular immotile spermatozoa for ICSI has so far resulted in good fertilization rates. However, to date only one case report has been published, describing a pregnancy and birth after transfer of thawed embryos (Cayan et al., 2001).

This case report is the first where pregnancies were achieved in fresh IVF cycles of couples with male KS, where immotile testicular spermatozoa were used for ICSI. It is also the first report where a 50/50 split between ejaculate and testicular sperm has been performed in the same IVF cycle. In the first case no fertilization occurred when vital HOS-tested ejaculated spermatozoa were injected but in the subsequent cycle, where viable HOS-tested testicular spermatozoa were injected, 75% of the oocytes were fertilized. In the second case, where both ejaculated and testicular HOS test positive spermatozoa were injected in the same IVF cycle, sperm from both origins resulted in acceptable fertilization rates. This couple was concerned about the multiple pregnancy risk and, as part of our not show any microdeletions. He had two brothers, one with ICS including recurrent airway infections and immotile sperm but no situs inversus.

The female partner was 35 years old and healthy. After two previous children from a donor insemination programme, the couple were interested in the IVF/ICSI programme to conceive a child with the patient’s sperm.

Before treatment the couple underwent genetic counselling for ICS/KS and genetic issues concerning testicular failure. Institutional review board approval was not required to proceed with therapy.
clinical policy; only one high quality embryo was transferred. In both case reports presented, all the transferred embryos had been fertilized with testicular sperm and the implantation rate is so far 100%.

The question arises why usage of ejaculated spermatozoa for ICSI seems more unpredictable compared with testicular spermatozoa. Cayan and co-workers reported that the probability of injecting non-vital spermatozoa was greater when ejaculated sperm was compared with that of testicular origin. Eosin-Y vital stain performed in a KS patient with totally immotile sperm revealed <5% and 95% viable sperm respectively (Cayan et al., 2001). In the first cycle in our first case fertilization failure occurred. However, all injected ejaculated spermatozoa were HOS-test positive which indicates membrane functionality but not necessarily fertilizing ability. Dead sperm are normally not present within the testes but dilution and centrifugation of minced testicular tissue after TESA is detrimental. Therefore, the HOS test is still of value to avoid usage of non-vital spermatozoa for ICSI.

Sperm analysis with Eosin-Y staining in combination with HOS of ejaculated spermatozoa might be of interest for testing sperm vitality in the first case with fertilization failure. However, this was not carried out due to the low sperm count in this patient and the lack of experience with the Eosin-Y test in our centre.

The long transit time for spermatozoa in the epididymides has earlier been suggested to increase the risk of senescent sperm degeneration. These aged spermatozoa contain fragile DNA, and pronucleus formation is subsequently hampered. Microtubule organization defects are known to result in failure to fertilize and an intensified disorganization is possible during the transit time in the epididymides. ICS/KS is a genetically heterogeneous condition related to ultrastructural defects of cilia. More than 100 different polypeptides have been identified in the constitution of the cilia, with the potential that a genetic mutation in genes coding for any of these proteins could result in the condition. It is an autosomal recessive condition and the chance that the female partner could be a carrier for a mutation in the same gene causing the male to be affected with risk of an affected offspring would be low. However, all patients must be informed about these possible risks and therefore genetic counselling is highly recommended to all couples prior to ICSI treatment.

The results of these case reports demonstrate that HOS-test of both testicular and ejaculated immotile spermatozoa is suitable and recommended before ICSI in men with ICS and KS. High fertilization rates and pregnancies were achieved after ICSI with immotile testicular spermatozoa. Usage of only immotile ejaculated sperm for ICSI, even if tested as viable, seems more unpredictable in fertilization outcome, which is in accordance with other published case-reports. The reason for this difference remains unclear and we welcome further comparative studies to confirm this tendency and to clarify the mechanism. Meanwhile, we recommend at least 50% of ICSI to be conducted with testicular immotile spermatozoa in couples with male ICS/KS to minimize the risk for total fertilization failure.

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References


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