CASE REPORT

Kartagener's Syndrome and Intracytoplasmıc Injection of Ejaculated Immotile Spermatozoa Selected by Hypo-Osmotıc Swelling Test

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Abstract

Kartagener's syndrome (KS) is a rare disorder which is defined as *situs inversus* accompanying defective cilial ultrastructure in ciliated cells and spermatozoa. Intracytoplasmic sperm injection is the treatment option for fathering a child in these cases. A few succesful pregnancies and healthy births have been reported in cases with KS treated with intracytoplasmic sperm injection using immotile spermatozoa. Here we present two cases of KS, succesfully treated by intracytoplasmic sperm injection combined with hypo-osmotic swelling test. In both cases, normal fertilization rates were achieved by using ejaculated immotile spermatozoa choosen by hypo-osmotic swelling test for the identification of the viable spermatozoa and in the first case, two cycles resulted in a healthy birth.

Keywords: intracytoplasmic sperm injection, Kartagener's syndrome, hypo-osmotic swelling test, asthenozoospermia

Özet

Kartagener Sendromu ve Ejakülatta Hipo-ozmotik Şişme Testi ile Seçilen İmmotil Spermatozoanın İntrasitoplazmik Enjeksiyonu

Kartagener sendromu, silialı hücrelerde ve spermatozoada ultrastrüktürel bozukluğa eşlik eden *situs inversus* olarak tanımlanır ve nadir görülen bir durumdur. Çocuk isteği olan hastalarda intrasitoplazmik sperm enjeksiyonu ile tedavi mümkündür. Kartagener sendromunda immotil spermatozoanın kullanıldığı intrasitoplazmik sperm enjeksiyonu vakalarında gebelik ve canlı doğum bildirilmiştir. Bu makalede, Kartagener sendromu olan iki hastada hipo-ozmotik şişme testi ile kombine intrasitoplazmik sperm enjeksiyonu tedavisi anlatılmıştır. Her iki vakada da, intrasitoplazmik sperm enjeksiyonunda sperm seleksiyonunda ejakülattaki immotil spermatozoaya hipo-ozmotik şişme testi uygulanmış ve canlı spermatozoa kullanılmıştır. İki hastada da, normal fertilizasyon oranları elde edilmiş ve iki siklus sonrası ilk hasta sağlıklı bir bebek dünyaya getirmiştir.

Anahtar sözcükler: intrasitoplazmik sperm enjeksiyonu, Kartagener sendromu, hipo-ozmotik şişme testi, astenozoospermi

Introduction

Immotile cilia syndrome is defined as defective cilial ultrastructure in ciliated cells and spermatozoa. The disease has an autosomal recessive inheritance with an incidence of one in 20 000 live births (1). Clinical presentation of the syndrome includes respiratory tract infections, bronchiectasis and male infertility. The dynein arms connecting the microtubules are abnormal or absent which is the cause for sperm immobility and ciliary epithelial disfunction. The disorder is referred to as Kartagener's syndrome when situs inversus accompanies immotile cilia syndrome (2).

Couples in whom the male partner has immotile cilia syndrome or Kartagener's syndrome are inevitably sterile due to immotility of the spermatozoa and the chance of achieving a spontaneous pregnancy is almost impossible. Although a few pregnancies using subzonal sperm injection (SUZI) or *in vitro* fertilization (IVF) have been reported in patients with Kartagener's syndrome (3-5), currently intracytoplasmic sperm injection (ICSI) is the only treatment option to be offered for these couples. But even using ICSI, as immotile ejaculated spermatozoa were used, total absence of fertilization or very low fertilization and pregnancy rates have been reported

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(1,6,7). Recently, however, it has been observed that the use testicular spermatozoa instead of ejaculated ones or selecting viable spermatozoa from ejaculate by hypo-osmotic swelling (HOS) test increase fertilization and pregnancy rates (1,6,8). In this report, we present two cases of Kartagener's syndrome succesfully treated by ICSI combined with hypo-osmotic swelling (HOS) test. In both cases, normal fertilization rates were achieved by using ejaculated immotile spermatozoa selected by hypo-osmotic swelling test for the identification of the viable spermatozoa and ICSI resulted in a healthy birth in the first case.

Case 1

A 27-years old woman and her 33-years old husband were admitted to our IVF clinic with primary infertility for the last six years. Her medical history was uneventful. Her cycles were regular and transvaginal ultrasound examination was normal. The hysterosonography demonstrated a normal uterine cavity. The male partner had a history of chronic bronchitis and *situs inversus*. The urological examination of the male was normal. Semen analysis revealed; a sperm concentration of $28x10^6$/ml without any motility. Two percent of spermatozoa were morphologically normal using Kruger strict criteria (9). Vitality assessment with eosin-Y test showed 50% vital spermatozoa in the ejaculate.

After counselling the couple about IVF/ICSI cycles, controlled ovarian hyperstimulation with 200 IU of recombinant FSH (rFSH, Puregon, Organon, Netherlands) was initiated following gonadotropin releasing hormone analogue (Lucrin, Abbott, Switzerland) down regulation. Gonadotropin dosage was tailored according to patient's ovarian response. When at least three follicles reached 18 mm in diameter, ovulation was induced by intramuscularly administered 10 000 U of hCG (Pregnyl, Organon, Netherlands) and oocytes were retrieved 35 hours after hCG injection. Of the 12 oocytes obtained, 11 were metaphase II and suitable for ICSI. Sperm concentration was $50x10^6$/ml in the ejaculate and all were immotile. The samples were prepared using one-layer gradient centrifugation (Suprasperm 90%, Medicult, Denmark). After two final washings (G-sperm, Vitrolife, Sweden), the morphologically normal spermatozoa were selected and resuspended to a desired volume of G-sperm solution mixed with sterile penta-distillated tissue culture grade water 1/1 dilution in order to select the viable spermatozoa (HOS test). After 10 minutes of incubation, viable tail swollen spermatozoa were individually selected and placed in the ICSI dish, washed in fresh G-mops culture media (Vitrolife, Sweden) drops and the ICSI procedure was performed by aggresive sperm immobilization (10).

The evaluation of oocytes 16-18 hours after injection revealed nine normally fertilized oocytes with two pronuclei. Three days after oocyte retrieval, four grade 1 embryos (three at the 7-cell and one at 8-cell stage) were transferred under abdominal ultrasound guidance. Luteal phase was supported by vaginally administered 600 mg/day micronized progesterone (Progestane, Koçak, Istanbul, Turkey). β-hCG testing was negative 12 days after embryo transfer.

Two years later, the couple decided to undergo another ICSI cycle. In the second cycle with the same ovulation induction protocol, 17 oocytes were retrieved out of which 14 were mature. The semen analysis on the day of oocyte pick-up showed $100x10^6$/ml concentration, 1% normal morphology and totally immotile spermatozoa. After sperm preparation all spermatozoa were still immotile and hypoosmotic swelling test was performed to discriminate live spermatozoa as described previously. Ten of the MII oocytes subjected to ICSI were normally fertilized. On day 3, four embryos (three grade 1 at 8-cell and 9-cell stage and one grade 2 at 8 cell stage) were transferred. Three weeks after a positive β-hCG test, a singleton clinical pregnancy was confirmed by transvaginal ultasound examination with a fetal heart beat. The uncomplicated pregnancy resulted in the birth of a healthy male infant with a 3360 gr weight at 37 gestational weeks.

Case 2

A 35-years old man was admitted to our clinic with a diagnosis of Kartegener's syndrome and primary infertility of seven years duration. His medical history revealed recurrent respiratory tract infections, bronchiectasis and a total situs inversus. The female partner was 28 years old and had a normal gynecological examination. The urological evaluation of the male partner was normal. The semen analysis revealed a sperm concentration of $52x10^6$/ml and no evidence of sperm motility. Morphological analysis showed 1% normal spermatozoa using Kruger strict criteria. Sperm vitality assessment with eosin-Y test showed 70% vital spermatozoa in the ejaculate.

The couple was counselled about the treatment procedure and assisted reproduction technologies. The female partner underwent controlled ovarian hyperstimulation after down regulation with GnRH analogue (Lucrin, Abbott, Switzerland). Ovarian stimulation was started on day 3 of cycle with 200 IU recombinant FSH (rFSH, Puregon, Organon, Netherlands). When at least three follicles reached 18 mm in diameter, ovulation was induced by intramuscularly administered 10 000 U of hCG (Pregnyl, Organon, Netherlands). Fifteen oocytes were retrived and 12 were at MII. Sperm concentration was $190x10^6$/ml, all spermatozoa were immotile and 1% were morphologically normal. After selecting the spermatozoa by HOS test, ICSI was carried out and fertilization was evaluated 16-18 hours later. Seven of the twelve injected oocytes were normally fertilized. Three days after oocyte retrieval, two grade 1 embryos (both at 6-cell) and two grade 2 embryos (one at 9-cell and one at 5-cell stage) were transferred under abdominal ultrasound guidance. Luteal phase was supported by vaginally administered 600 mg/day micronized progesterone (Progestane, Koçak, Istanbul, Turkey). β-hCG testing 12 days after embryo transfer was negative.

Discussion

The couples in whom the male partner suffers from Kartegener's syndrome are considered to be invariably infertile due

to total immotility of spermatozoa. Currently, the only treatment option for these patients is ICSI. Although, ICSI bypasses the natural mechanisms for fertilization and overcomes the factors related to impaired motility, the success of micromanipulation in the treatment of patients with Kartagener's syndrome is unpredictable as the viability of immotile spermatozoa are not known. Von Zumbusch et al. (7) reported two cases of ICSI using immotile ejaculated spermatozoa resulting in 67% fertilization and the birth of healthy male/female twins in one and 50% fertilization and the birth of a healthy female in the other. Fonttis et al. (11) also reported a fertilization rate of 40% and a pregnancy in one further case. But, total failure of fertilization have been reported in three cases with Kartagener's syndrome where ejaculated immotile spermatozoa were used (4,6,12). Therefore, the use of immotile spermatozoa without the knowledge of viability of selected sperms for ICSI is the major concern in these cases and the reported total fertilization failures described above can be explained by incidental use of non-viable spermatozoa for ICSI.

The use of testicular spermatozoa rather than ejaculated spermatozoa for ICSI or using hypoosmotic swelling test for selection of viable spermatozoa were suggested to improve fertilization and ongoing pregnancy rates in these cases. Çayan et al. (1) used surgically retrieved testicular spermatozoa for ICSI combined with HOS test in one case with Kartagener's syndrome and testicular spermatozoa without HOS test in another case with immotile cilia syndrome. They achieved a fertilization rate of 63% and 60%, respectively, and a pregnancy resulting in the birth of a normal healthy girl in the former case. They concluded that, with testicular sperm, successful fertilization after ICSI had been possible despite the lack of sperm motility in couples with Kartagner/immotile cilia syndrome. Westlander and colleques (6) reported three ICSI cycles in two cases with Kartagener's syndrome, in which viable ejaculated or testicular spermatozoa were selected using hypoosmotic swelling test. In case 1; total fertilization failure occurred in the first cycle in which ejaculated spermatozoa were used, whereas the second cycle, using testicular spermatozoa, resulted in 75% fertilization rate and delivery of healthy twin babies. In case 2; half of the oocytes were injected with ejaculated and half with testicular spermatozoa and fertilization rates of 44% and 56% were achieved respectively. High quality embryos were obtained in both groups and the transfer of a single embryo derived from testicular spermatozoa resulted in a singleton pregnancy. Recently, in trials on a couple with immotile cilia syndrome, a pregnancy obtained in a fresh cycle which was terminated as a late miscarriage at 21 weeks of gestation and a subsequent ongoing pregnancy achieved by the transfer of frozen-thawed embryos obtained by the ICSI of HOS-tested immotile spermatozoa were reported (8). A few similar cases have also been reported (13,14). Yet, using the HOS-tested ejaculated immotile spermatozoa for ICSI does not guarantee fertilization and fertilization failure does occur (6).

As stated previously, to overcome the low fertilization and pregnancy rates using immotile spermatozoa for ICSI (15), the suggested strategies are the use of testicular spermatozoa (1,6,16) or ejaculated spermatozoa after HOS test. The HOS test has proved to be valuable for increasing fertilization, cleavage and pregnancy rates in ICSI cycles where no motile spermatozoa were recovered in cases of so called complete asthenozoospermia (17,18). The HOS test is a simple, practical and and reliably repeatable test without any toxicity as physiological substances are used. It allows the selection of viable spermatozoa prior to oocyte injection. HOS solution is a hypo-osmotic solution and its use during sperm preparation causes a hypo-osmotic reaction in viable immotile spermatozoa; the flagellum curls and starts to swell, whereas no reaction at all is seen with dead spermatozoa (8).

As far as we know, only two births have been achieved after using HOS-tested ejaculated spermatozoa in cases with Kartagener's syndrome or immotile cilia syndrome (1,8). Here, we have reported the results of 3 ICSI cycles in two cases with Kartaganer's syndrome. In all cycles, ejaculated immotile spermatozoa were used after selecting the viable spermatozoa by HOS test. The fertilization rates were 81% and 71% in two cycles of the first couple and 58% in the second couple. The second cycle of the first case resulted in the birth of healthy male infant. In conclusion, we have suggested that the selection of viable spermatozoa by HOS test in cases with a total absence of sperm motility due to Kartagener/immotile cilia syndrome has apparently obviated the necessity to use the testicular spermatozoa and provided high fertilization and acceptable pregnancy rates.

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