CASE REPORT

High fertilization rate in conventional in-vitro fertilization utilizing spermatozoa from an oligozoospermic subject presenting microdeletions of the Y chromosome long arm

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A case is reported in which a high fertilization rate was achieved by conventional in-vitro fertilization (IVF), using spermatozoa from an oligozoospermic man carrying a microdeletion of the long arm of the Y chromosome. The patient presented with idiopathic infertility of 10 years duration; the fertility status of his wife was completely normal. After IVF, five out of eight oocytes retrieved showed normal fertilization and four showed normal embryo cleavage. Four embryos were transferred; however, pregnancy did not result. These results demonstrate that spermatozoa from oligozoospermic patients carrying a Yq microdeletion are fully competent in achieving capacitation, acrosome reaction and fertilizing ability during IVF. Therefore, although definitive conclusions cannot be made from a single case report, we suggest that Yq microdeletion analysis should be considered in oligozoospermic patients undergoing conventional IVF.

Key words: case report/infertility/IVF/oligozoospermia/Y-chromosome

Introduction

Deletions in the Y chromosome long arm (Yq) are known to represent the pathogenetic mechanism for a small number of infertile men, especially in cases of severe testiculopathies (Sertoli cell-only syndrome and severe hypospermatogenesis) (Reijo et al., 1995, 1996; Kent-First et al., 1996; Vogt et al., 1996; Foresta et al., 1997, 1998; Pryor et al., 1997; Vereb et al., 1997). To date, two gene families have been identified in Yq as fundamental for normal spermatogenesis, and have been defined as RBM and DAZ (Ma et al., 1993; Reijo et al., 1995). Deletions in both gene families have been detected in idiopathic azoospermic and severely oligozoospermic patients. However, there is increasing evidence that regions in Yq other than RBM and DAZ may determine the same tubular alterations (Kent-First et al., 1996; Stuppia et al., 1996; Vogt et al., 1996; Foresta et al., 1997, 1998; Pryor et al., 1997). Furthermore, similar Yq deletions may be present in subjects either with oligozoospermia or azoospermia, highlighting the lack of a phenotype-genotype relationship. Recently it has been demonstrated that spermatozoa from Yq-deleted patients are fully competent in fertilizing the oocyte after intracytoplasmic sperm injection (ICSI), and in achieving pregnancy (Mulhall et al., 1997). In addition, there are some reports demonstrating that spermatozoa carrying Yq deletions can also fertilize in vivo (Stuppia et al., 1996; Vogt et al., 1996; Pryor et al., 1997). In the present case report we describe the success of conventional IVF using spermatozoa from an oligozoospermic subject presenting with a Yq deletion.

Case report

The patient was a 39 year old man married to a 34 year old woman and complaining of infertility for 10 years. The patient showed no gonadal abnormalities, varicocele and had no history of cryptorchism, post-mumps orchitis, cancer chemotherapy, irradiation, testicular trauma or other causes of possible testicular damage. The female partner was examined and found to have normal ovulatory cycles (confirmed by ultrasound examination of the ovaries during the ovulatory phase and ovulatory progesterone plasma concentrations at day 21), normal hysterosalpingography and absence of sperm antibodies in the cervical mucus and serum.

Two different semen analyses, separated by a 3 week interval, following a 3 day period of sexual abstinence, were performed according to World Health Organization guidelines for semen analysis (WHO, 1992). The sperm concentration was 3.7×10^6 and 5.3×10^6 cells/ml, with a total of 9.0×10^6 and 10.6×10^6 cells/ejaculate; viability, as demonstrated by the eosin test, was 70 and 64% with percentage motility as follows: grade A = 17 and 10%; grade B = 23 and 28%; grade C = 4 and 5%; grade D = 56 and 57%; normal morphology was 41 and 35%.

Ultrasound scanning of left and right testes demonstrated a volume of 9 and 13 ml respectively, without any pathological findings. The basal follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone plasma concentrations were 5.8, 3.8 IU/l and 14.9 nM respectively.

The testicular structure was evaluated by bilateral testicular fine needle aspiration cytology, as previously described by Foresta and Varotto (1992). Cytological analysis showed the
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Figure 1. Sequence-tagged sites (STS)-polymerase chain reaction (PCR) data of Yq microdeletions detected in the patient lymphocytes and spermatozoa. Deletion intervals and Y-chromosomal STS used are listed above. Results for fertile men are shown for comparison. Black box = STS present; white box = STS absent.

Microdeletions in the Yq were studied in the DNA extracted from peripheral lymphocytes by polymerase chain reaction (PCR) using a set of 29 sequence-tagged sites (STSs), spanning the whole euchromatic region, following the method and the STSs localization previously described (Foresta et al., 1997).

In all, 20 healthy normal men of proven fertility and 10 normal women have been previously studied as positive and negative controls respectively. In each polymerase chain reaction (PCR) experiment a sample of DNA from positive and negative controls were included. The patient was considered positive for a STS if the PCR product was of the expected size and negative only after three amplification failures. Using these criteria we detected a Yq microdeletion in interval 5 and a smaller deletion in interval 6D, outside both RBM and DAZ gene families (Figure 1). All normal men were previously found to carry an intact Yq.

The patient’s father was not available and he has no brothers, but on the basis of previous findings (Reijo et al., 1995; Foresta et al., 1997, 1998) the Yq deletion may be considered as the cause of the testicular damage. The couple, informed about the possible risk of transmitting this abnormality to male children, gave their consent for IVF.

Ovarian stimulation

Ovarian stimulation was carried out by administering a gonadotrophin-releasing hormone (GnRH) analogue (triptorelin, Decapeptyl 3.75; Ipsen, Paris, France) at day 21 of the cycle preceding that of ovarian stimulation with FSH (Metrodin; Serono, Milan, Italy) administered from cycle day 3; follicular growth was monitored by daily vaginal ultrasound and measuring serum oestradiol starting after 9 days of FSH treatment. Human chorionic gonadotrophin (HCG 10 000 IU, Profasi; Serono, Milan, Italy) was administered when the cohort of follicles reached a diameter >18 mm. We recovered eight oocytes by transvaginal aspiration 36 h after HCG administration and transferred them to culture wells containing human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA, USA), with 0.5% human serum albumin at 37°C in controlled atmosphere (CO₂ 5% and O₂ 95%).

Sperm collection and preparation

After oocyte retrieval, a semen sample was collected by masturbation and allowed to liquefy at room temperature. After liquefaction seminal standard parameters were determined as described above. Spermatozoa were selected by discontinuous Percoll gradients utilizing HEPES-buffered HTF medium supplemented with 0.5% human serum albumin (HSA) (Irvine Scientific). After selection, spermatozoa were suspended in modified HTF medium supplemented with 0.5% HSA and incubated at 37°C in controlled atmosphere until use.

IVF procedure

After sperm selection, 100 000 motile spermatozoa were added to each one of eight culture wells, which each contained one oocyte. Fertilization was evaluated after 16–20 h and the presence of five oocytes with two pronuclei with a fertilization rate of 62.5% (five out of eight) was noted. Cleavage was checked after 46 h and the presence of four embryos at the 2-cell stage was observed. Embryo transfer was performed on day 2 after oocyte retrieval utilizing a Frydman’s catheter (Laboratoire CCD, Paris, France) for uterine transfer. The luteal phase was supported by administering progesterone (50 mg i.m. daily, Gestone; AMSA, Italy) starting from the day of embryo transfer. A serum β-HCG test was performed 11–15 days after the transfer and it was repeatedly negative.
Discussion

It has recently been demonstrated that the pathogenesis of the testicular infertility in a high percentage of severe oligozoospermic and azoospermic patients may be related to microdeletions of the Y chromosome long arm (Yq) (Reijo et al., 1995, 1996; Kent-First et al., 1996; Vogt et al., 1996; Foresta et al., 1997, 1998; Pryor et al., 1997), and in previous studies we have observed that Yq microdeletions in highly selected idiopathic severe primary testicularopathies, e.g. severe hypospermato genesis and Sertoli cell-only syndrome, were present in five of 22 subjects (22.7%) and 10 out of 18 subjects (55.5%) respectively (Foresta et al., 1997, 1998). ICSI allows fertilization and pregnancy in the presence of severe oligozoospermia and azoosper mia utilizing few ejaculated, epididymal or testicular spermatozoa. The possible risks of transmitting genetic abnormalities utilizing this technique have been proposed only very recently, since ICSI bypasses all the physiological mechanisms related to fertilization (Foresta et al., 1996a,b). ICSI utilizing spermatozoa from these Yq deleted patients will transmit this defect to male children, as recently reported by Kent-First et al. (1996). However, it has been noted that it is possible to have a deletion in the Y chromosome and to father children (Stuppia et al., 1996; Vogt et al., 1996; Pryor et al., 1997). On the other hand the origin of Yq deletions is still unknown and only speculative hypotheses can be formulated, as excellently reported recently by Edwards and Bishop (1997). Mulhall et al. (1997) have recently reported fertilization and pregnancy achieved utilizing ICSI with testicular spermatozoa from azoospermic patients presenting deletions in the DAZ region of Yq suggesting that Yq microdeletions are compatible with the presence of fully competent spermat ozoa. The present report is the first showing that spermatozoa from an oligozoospermic subject presenting Yq microdeletions are able to fertilize oocytes in vitro. These findings undoubtedly demonstrate that spermatozoa carrying Yq deletions possess all the characteristics required for regulating capacitation, acrosome reaction and the ability to penetrate the oocyte and fertilize it, as previously showed also in vivo (Stuppia et al., 1996; Vogt et al., 1996; Pryor et al., 1997).

PCR analysis, as previously reported by our and other, groups (Najmabadi et al., 1996; Foresta et al., 1997, 1998), showed the presence of interstitial double deletions, that can be explained by different hypotheses: (i) the PCR observations may reflect separated microdeletions; (ii) some STSs may be from repetitive sequences and PCR products may reflect amplifications from a different site; (iii) a complex rearrangement may be the cause (e.g. an inversion in the father who would still be karyotypically normal with a subsequent interstitial deletion). Discontinuous selections seem to occur frequently in the Y chromosome (Najmabadi et al., 1996; Foresta et al., 1997, 1998; Girardi et al., 1997), probably because it contains a high frequency of repetitive elements in tandem repeats, rendering this chromosome highly unstable (Girardi et al., 1997). The Yq deletion found in our patient is located outside both DAZ and RBM genes, suggesting that regions other than these loci may be associated with spermatogenic impairment, according to recent studies (Stuppia et al., 1996; Kent-First et al., 1996; Vogt et al., 1996; Foresta et al., 1997, 1998; Pryor et al., 1997). This deletion partially overlaps previously described regions in intervals 5 and 6 of Yq (AZFa,b,c) (Vogt et al., 1996): in particular, the distal deletion in interval 6D lies outside the DAZ gene and encompasses two STS loci; the proximal deletion encompasses 12 STS loci from intervals 5A to 6A, outside the RBM gene and overlaps AZFa entirely and AZFb partially. The functions of the Yq regions deleted in this patient are not yet known, but recent data suport the idea that they harbour one or more genes for male germ cell development, above all in Yq interval 5 (Vogt et al., 1996; Foresta et al., 1998). Unfortunately, no male relatives were available and thus the Yq deletions cannot be absolutely associated to the testicular damage. However, on the basis of previous reports we can cautiously make this association.

These data demonstrate that the rate of fertilization and embryo development in our Yq deleted subject are similar to that obtained in normozoospermic subjects undergoing IVF because of female factor. Therefore, it appears that the possible transmission of a Yq deletion from father to son can occur not only with ICSI but also with conventional IVF techniques. Even if data are lacking on the prevalence and consequences of Yq deletions in oligozoospermic men and although definitive conclusions cannot be made from a single case, we suggest that Yq microdeletion analysis not only be considered in oligozoospermic patients undergoing ICSI but also when undergoing other IVF programmes.

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References


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