CASE REPORT

Birth of a healthy newborn following the intracytoplasmic injection of testicular spermatozoa from a patient with Klinefelter’s syndrome

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Klinefelter’s syndrome is one of the known causes of azoospermia or cryptozoospermia, and it may present in non-mosaic (47,XXX) or mosaic (47,XXX/46,XY) form. The likelihood of finding spermatozoa in the ejaculate or testicular tissue of patients with mosaic Klinefelter’s syndrome is low, and with the non-mosaic form, even lower. We describe a patient with non-mosaic Klinefelter in whom initially non-motile spermatozoa were derived from searching the ejaculate. Ten mature oocytes were injected, but none was fertilized. Subsequently, testicular biopsy was undertaken in order to collect spermatozoa for oocyte injection. Fifteen motile sperm cells were found and injected. Nine oocytes were fertilized and cleaved; three embryos were transferred into the uterine cavity. The woman conceived and following a normal pregnancy delivered a healthy child. Genetic analysis of the neonate disclosed a normal 46,XY karyotype. Non-motile spermatozoa in the ejaculate did not prove their fertilization potential, but their presence did not exclude finding motile, fertile spermatozoa in the testicular tissue in a non-mosaic Klinefelter patient. This report is further evidence that normal spermatozoa with fertilization potential are produced in the testes of patients with Klinefelter’s syndrome.

Key words: 47,XXX/azoospermia/intracytoplasmic sperm injection/Klinefelter’s syndrome/testicular biopsy

Introduction

Klinefelter et al. (1942) described a syndrome consisting of gynaecomastia, hypogonadism and male infertility due to a 47,XXX karyotype. This situation may be found in one of 500 newborn males (Nielson and Wohlert, 1991) and in 3.1% of the infertile male patients (Guichaoua et al., 1993). Patients with azoospermia and non-mosaic 47,XXX Klinefelter’s syndrome were considered infertile in the past. Occasionally, a patient with chromosomal mosaicism consisting of 46,XY/47,XXX karyotype will have fertile spermatozoa in their ejaculate. Now, with the advent of testicular sperm extraction and intracytoplasmic sperm injection (ICSI), patients with Klinefelter’s syndrome may realize their reproductive potential.

To date, four pregnancies have been reported in two centres where the husband in each couple had non-mosaic Klinefelter’s syndrome (Tournaye et al., 1997; Palermo et al., 1998). All of them resulted in deliveries of four healthy infants. We describe the fifth birth of a normal infant to a father with non-obstructive azoospermia due to non-mosaic Klinefelter’s syndrome.

Case report

A 24 year old man and his 21 year old spouse were referred to our in-vitro fertilization (IVF) programme in January 1997 after 1 year of primary infertility. Physical examination revealed normal appearance (height 173 cm, weight 61 kg) and normal hair distribution. Gynaecomastia was not seen. Testicular volume was estimated respectively to be 6 ml each testis. Six semen analyses with an average volume of 3.2 ± 1.3 ml showed no spermatozoa. Centrifugation of three semen samples revealed 3–21 immotile sperm cells in the pellet, most of them with abnormal morphology. Blood analysis demonstrated an elevated concentration of follicle stimulating hormone (FSH) of 25.9 IU/L, luteinizing hormone (LH) 11.8 IU/L, low testosterone 12.2 µg/l and normal prolactin, 318 pmol/l.

Peripheral blood chromosome analysis of 26 cells showed a 47,XXX karyotype in 24 cells, 45,XXX,-15,-21 in one cell and 42,XXX,-6,-17,-20,-21,-22 in an additional cell. A second peripheral blood chromosome analysis was performed in a different medical centre on 16 cells. All cells showed a 47,XXX karyotype. A buccal smear was performed and found X chromatin in 9% of the cells, which relates to our findings in normal female (46,XX) patients. These chromosome analyses led us to the conclusion that this patient has the non-mosaic form of Klinefelter’s syndrome.

His wife had regular ovulatory cycles, and a normal hysterosalpingogram. Ovarian stimulation was achieved by the combination of gonadotrophin releasing hormone (GnRH) agonist (Decapeptyl®, microcapsules, 3.75 mg; Ferring, Malmo, Sweden) and human menopausal gonadotrophin (Pergonal®, Teva, Petah-Tiqua, Israel). Human chorionic gonadotrophin (HCG; Chorigon®, Teva), 10 000 units, was administered when the leading follicle reached a mean diameter of 21–22 mm diameter and oestradiol concentration was 2800 pg/ml. Oocyte collection was 33–35 h later and the luteal phase was supplemented with progesterone in oil (Gestone®, Pains & Byrne, West Byfleet, Surrey, UK), 50 mg per day i.m.

The cycle of treatment was initiated in February 1997, when 13 oocytes were collected; 10 metaphase II (MI) oocytes...
were used for ICSI with retrieved immotile spermatozoa from the ejaculate, found after an extended sperm preparation (ESP) (Ron-El et al., 1997). Sperm morphology was normal as far as could be observed under an inverted microscope (Hoffman Modulation Optics Inc., Greenvale, NY, USA) (×400). None of the 10 injected oocytes fertilized and only two of them demonstrated extrusion of a second polar body.

In view of the results of the first treatment, a second trial was suggested using testicular spermatozoa achieved by testicular biopsy. The woman was prepared with the same ovarian stimulation protocol. Testicular biopsy was performed in April 1997. After ESP again revealed few immotile spermatozoa, two biopsies were taken from the right testis under general anaesthesia. Immediate evaluation revealed non-motile spermatozoa. The wet preparation was elaborated as previously described (Friedler et al., 1997). Oocyte retrieval was carried out, with 23 mature oocytes collected; HCG administration was made when leading follicles were 21 mm and oestradiol was 2123 pg/ml.

Aliquots of the suspension from the testicular tissue homogenate were distributed after 3 h of incubation, in dozens of droplets, each of 8 µl, for a careful search for spermatozoa. Fifteen motile spermatozoa were found and injected into 15 MII oocytes. Nine of these oocytes fertilized; in one of them the two pronuclei were irregular in size and in another oocyte several vacuoles were present in addition to the pronuclei. An additional single pronucleated oocyte was visible and another two degenerated following their injection. All nine oocytes cleaved, three of which were replaced 72 h after oocyte retrieval at the 8-cell stage; these were judged to be of grade I morphology.

In an additional search for spermatozoa after another 24 h of incubation, 14 motile and nine non-motile spermatozoa were visible in 10 medium droplets. The remaining testicular tissue was frozen after removing a small specimen for histology. Histological findings were as follows: seminiferous tubules with thick membranes and sporadic sperm cells in different stages of spermatogenesis in the lumen with an oedematous stroma.

The couple consistently refused any preimplantation or prenatal genetic diagnosis which was extensively discussed on several occasions before starting their treatments.

Pregnancy was achieved, and the first βHCG concentration was 67 IU 2 weeks after the embryo transfer. A singleton pregnancy with visible heart pulsation could be seen in the sixth gestational week. The pregnancy was uneventful and in January 1998 the patient had a normal vaginal delivery at term of a healthy boy weighing 3660 g. A fluorescence in-situ hybridization (FISH) analysis in lymphocytes from the infant’s peripheral blood showed an XY karyotype. A further cytogenetic karyotype from the infant’s lymphocytes disclosed 22 normal paired somatic and one pair of XY chromosomes in all cells examined.

Discussion

Klinefelter’s syndrome is a disorder of the gonad due to an error in meiosis leading to an abnormal karyotype (Rothwell, 1993). Defective function of Leydig cells at onset of puberty will cause secretion of high oestradiol and low to normal testosterone concentrations, and so the LH and FSH will be elevated. In the testes, tubules become fibrotic and hyalinized, the tubule lumen will gradually obliterate and germ cells will disappear with time.

The incidence of spermatozoa carrying a 24,XY karyotype in normal men is 0.08 to 0.24% (Moosani et al., 1995), and in mosaic Klinefelter’s syndrome patients 2.09% as demonstrated by FISH (Chevret et al., 1995). The frequency of hyperploidy in the spermatozoa found in non-mosaic Klinefelter patients is still unclear.

Although non-mosaic 47,XXX patients demonstrate such a genetic disorder at high frequency in their lymphocyte culture, they may have mosaicism for 46,XY in other tissues, among them germ cells. The recent finding of normal karyotypes in embryos from three couples in which the father had a 47,XXX karyotype (Staessen et al., 1996) supports the hypothesis that abnormal X chromosome ‘dosage’ in germ cells causes the near-total incapability of sperm production or early sperm cell death at the spermatocyte stage (Harari et al., 1995; Muller et al., 1995). A recent study on a patient with mosaic Klinefelter’s syndrome showed a significantly different frequency of sex chromosome aberrations in spermatozoa from that found in his somatic cells: 7.5% hyperhaploidy in spermatozoa compared with 93.9% in peripheral lymphocytes (Kruse et al., 1998). Sperm hyperploidy was detected in only 2.7% of the spermatozoa of another non-mosaic Klinefelter patient (Hinney et al., 1997).

Therefore the probably relatively rare possibility exists that a specific spermatozoon injected into the oocyte can be hyperhaploid and fertilize. More studies are warranted to specify the relationship of the hyperhaploidy between germ and somatic cells in mosaic and non-mosaic Klinefelter patients.

Since embryo biopsy for preimplantation genetic diagnosis (PGD) is available, treatment by IVF–ICSI of a couple whose male partner suffers from non-mosaic Klinefelter’s syndrome is justified. The couple should be informed about the potential risk of using spermatozoa from a man with Klinefelter’s syndrome. In any case, the final decision regarding PGD should be made by the couple after a detailed explanation of the options.

The frequency of absolute azoospermia in the ejaculate of a patient with non-mosaic Klinefelter’s syndrome is unclear. According to previous studies, the frequency of finding spermatozoa in the ejaculate was 5.8% or 2.6% for such patients (Paulsen et al., 1968; Foss and Lewis, 1971 respectively). Recent studies showed seven out of 13 patients with non-mosaic Klinefelter’s syndrome having few mature spermatozoa in their ejaculate (Tournaye et al., 1997; Bourne et al., 1997; Hinney et al., 1997; Palermo et al., 1998; Kruse et al. 1998). Some motile spermatozoa were observed in the ejaculate in only one of these six cases, which were collected and frozen. Subsequent treatment by ICSI with this thawed spermatozoa led to fertilization, embryo formation and a pregnancy which resulted in the birth of a healthy child (Bourne et al., 1997). Five other patients who had only few immotile spermatozoa,
most of them with abnormal morphology, were directed to testicular sperm extraction (TESE).

In our case report, immotile spermatozoa were found after ESP of the ejaculate, and they were used for ICSI; however, not a single fertilization occurred among the 10 injected eggs. This questions the efficiency of utilizing immotile spermatozoa found in the ejaculate of an individual with Klinefelter’s syndrome. We have already experienced failure of fertilization in some patients with cryptozoospermia when using immotile spermatozoa for ICSI from their ejaculate. The current case also demonstrates that although immotile spermatozoa are present in the ejaculate, motile spermatozoa can be found in the testicular biopsies, with fertilizing potential.

Although possibly less efficacious, use of immotile spermatozoa from the ejaculate in these patients for ICSI is probably still warranted as a first line of treatment. If unsuccessful, testicular biopsy may then be utilized to collect motile spermatozoa for ICSI which may lead to fertilization, subsequent pregnancy and delivery of a normal infant.

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References


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