A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis

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Non-mosaic Klinefelter patients are generally azoospermic due to primary testicular failure. Nevertheless, in some cases, testicular spermatozoa may be recovered and utilized to fertilize oocytes via intracytoplasmic sperm injection (ICSI). As the risk for an increased number of gonosomes in these spermatozoa is unclear, preimplantation genetic diagnosis (PGD) may be attempted in the resulting embryos. In the present study, we report our experience with the combined approach of sperm retrieval by testicular fine needle aspiration (FNA), ICSI and PGD in seven consecutive non-mosaic Klinefelter individuals. In four patients, between one and five spermatozoa were retrieved in five out of nine consecutive attempts. In a fifth patient, only 10 round spermatids could be isolated. Mature spermatozoa were injected into a total of 16 metaphase-II oocytes, of which 11 (69%) remained intact. Two distinct pronuclei (2PN) were observed in four oocytes (36%) while a single pronucleus (1PN) was documented in two oocytes. Five cleavage stage embryos developed from the oocytes of two couples. Upon the request of one couple, their three embryos (two derived from 1PN oocytes) were transferred without PGD but pregnancy was not achieved. PGD by fluorescence in-situ hybridization (FISH) was performed in the two embryos of the other couple which were derived from normal fertilization. PGD results of one embryo were 18,18,X,X,Y, the embryo was not transferred and FISH analysis of the remaining blastomeres identified variable chromosome numbers in the nuclei. The second embryo was diagnosed as normal and was transferred, resulting in a successful pregnancy and birth. In conclusion, the results of this report indicate that a pregnancy and birth may be attained in azoospermic non-mosaic Klinefelter individuals by testicular FNA combined with ICSI. Due to the unknown risk of gonosomes aneuploidy in embryos from Klinefelter patients, PGD or prenatal diagnosis should be recommended.

Key words: FISH/ICSI/Klinefelter's syndrome/preimplantation genetic diagnosis/testicular spermatozoa

Introduction

Klinefelter's syndrome occurs in one out of 500 new-born phenotypic males. It is the most frequent chromosomal abnormality among infertile males, with an incidence of 3.1% (Guichaoua et al., 1993; Harari et al., 1995). Initially described by Klinefelter et al. (1942), the syndrome is characterized by gynaecomastia, small atrophic firm testes, azoospermia, and elevated levels of plasma gonadotrophins. The most common karyotype is 47,XXY; however, 46,XY/47,XXY mosaicism comprises about 10% of patients and additional karyotypic varieties have been described (Wilson and Griffin, 1987).

The majority of Klinefelter patients are azoospermic and sterile due to primary testicular failure. Although severe oligozoospermia has been infrequently observed, it has mostly been reported in cases with mosaic 46,XY/47,XXY karyotype (Gordon et al., 1972). Nevertheless, it seems that in some cases of non-mosaic 47,XXY Klinefelter's syndrome, a limited process of spermatogenesis may occur. There have been rare reports of severe oligozoospermia (Foss and Lewis 1971; Gordon et al., 1972) and even fatherhood following spontaneous pregnancies (Laron et al., 1982; Terzoli et al., 1992) or intracytoplasmic sperm injection (ICSI) (Bourne et al., 1997; Hinney et al., 1997). Furthermore, it was recently demonstrated that in some azoospermic 47,XXY Klinefelter individuals, testicular spermatozoa may be recovered and successfully utilized to fertilize oocytes via ICSI (Tournaye et al., 1996).

The risk of an increased number of gonosomes in spermatozoa which are retrieved from individuals with a 47,XXY karyotype is unknown. Cytogenetic studies of spermatozoa were mostly done in oligozoospermic mosaic 46,XY/47,XXY cases. In these individuals, an increased incidence of hyperhaploid 24,XY sperm cells has been demonstrated (Cozzi et al., 1994; Chevret et al., 1996; Martini et al., 1996). A single FISH analysis of sperm cells from an oligozoospermic, apparently non-mosaic, Klinefelter individual was recently reported and demonstrated an increased incidence of both 24,XY and 24,XX hyperhaploid gametes (Guttenbach et al., 1997). These results support the claim that 47,XXY germ cells may complete meiosis and produce mature hyperhaploid spermatozoa. Therefore, at present, it seems justified to attempt preimplantation genetic diagnosis (PGD) to rule out sex chromosome aneuploidy, in embryos of non-mosaic Klinefelter patients (Staessen et al., 1996).

Here, we report our experience with testicular fine needle aspiration (FNA), ICSI and PGD in seven non-mosaic

Table I. Patients' clinical characteristics

Patient no.	Age (years)	Karyotype (no. metaphases)	Beard	Gynaecomastia	Small testes	Serum FSH (1.4–18.1 IU/l)[a]	Serum LH (1.3–12.9 IU/l)[a]	Serum testosterone (10–35 nmol/l)[a]	No. spermatozoa at semen analysis[b]	Past semen analyses or micro-SEM analyses
1	29	47,XXY (30)	Present	Absent	Present	53.4	25.3	19	None (2)	10 non-motile spermatozoa in pellet, 10 months ago
2	35	47,XXY (31)	Scarce	Present	Present	41	32	12.5	None (2)	Motile spermatozoa in pellet at 27 years of age
3	25	47,XXY (30)	Scarce	Absent	Present	55	21	12.3	None (1)	8 spermatozoa in micro-SE
4	31	47,XXY (31)[c]	Scarce	Present	Present	18.5	12.6	6.6	None (2)	
5	37	47,XXY (30)	Present	Absent	Present	71.2	42.3	15	None (3)	
6	29	47,XXY (30)	Present	Absent	Present	28	NA	13.9	None (2)	9 spermatozoa in micro-SEM
7	27	47,XXY (30)	Scarce	Present	Present	NA	NA	NA	None (1)	

[a]Normal range.
[b]Semen analysis following centrifugation at 1800 *g*. Values in parentheses are numbers of semen analyses.
[c]One metaphase showed 48,XXXY.
FSH = follicle stimulating hormone; LH = luteinizing hormone; SEM = scanning electron microscopy.

Klinefelter patients and the achievement of a pregnancy and birth following this approach.

Materials and methods

Patients

Ten individuals with Klinefelter's syndrome were admitted to our IVF unit between 1995 and 1997. Cytogenetic analysis of at least 30 metaphases of peripheral blood lymphocytes was performed in each of the individuals. A mosaic 46,XY/47,XXY pattern was demonstrated in three patients. The other seven subjects in whom a non-mosaic 47,XXY karyotype was demonstrated were included in this study. In five patients, the non-mosaic pattern was further confirmed by FISH analysis of 100–200 peripheral blood lymphocytes. The findings of physical examination and the assessment of serum gonadotrophins and testosterone are summarized in Table I. All patients had facial hair; however, a scarce pattern was observed in four. Gynaecomastia was found in three patients, while all had small atrophic scrotal testes. Serum follicle stimulating hormone (FSH) and testosterone levels were available in six patients. Serum FSH levels were elevated in all, while serum testosterone levels were in the low normal range in five patients and decreased in one. Serum luteinizing hormone (LH) was increased in four patients, in the upper normal range in one individual and not available in two. Before being scheduled for testicular sperm retrieval, one to three sperm samples from each of the patients were carefully evaluated for the presence of sperm cells following high speed centrifugation (1800 *g*). None of these evaluations showed any spermatozoa. In two patients spermatozoa were demonstrated in past semen analyses, and in another two individuals, spermatozoa were demonstrated in the ejaculate by scanning electron microscopy (Table I).

The average age of the wives of these patients was 26.2 years (range 21–35 years), all having an unremarkable hormonal profile and a normal mechanical evaluation.

Ovulation induction

Controlled ovarian stimulation was induced by a desensitization protocol using a gonadotrophin-releasing hormone agonist (nafarelin,

Synarel; Teva, Petach Tikva, Israel) in association with human menopausal gonadotrophin (HMG, Pergonal; Teva) and human chorionic gonadotrophin (HCG, Chorigon; Teva) (Benshushan *et al.*, 1993). The luteal phase was supported with progesterone vaginal tablets (Dizengoff Pharmaceuticals, Tel-Aviv, Israel), 50 mg twice daily, supplemented with HCG 2500 IU on days 4, 8 and 12 post-embryo transfer.

Oocyte retrieval and preparation

Oocyte retrieval was carried out under transvaginal ultrasound guidance, 36 h after HCG administration. The cumulus–corona–oocyte complexes were incubated in human tubal fluid medium (HTF; Irvine Scientific, CA, USA) supplemented with 7.5% synthetic serum substitute (SSS; Irvine Scientific). Following 2 h incubation, the surrounding cumulus and corona cells were removed using 60 IU/ml hyaluronidase (type IV-S; Sigma, St Louis, MO, USA) in HEPES-enriched HTF medium (Irvine Scientific). Nuclear maturation was assessed under an inverted microscope and only metaphase II oocytes were microinjected.

Sperm retrieval by testicular fine needle aspiration

Testicular fine needle aspiration (FNA) was performed under general anaesthesia as previously described (Lewin *et al.*, 1996). The aspiration apparatus included 21 or 23 gauge butterfly needles connected to a 20 ml syringe installed in an aspiration handle (Cook, Queensland, Australia). The butterfly needle was introduced into the testes and steady suction was established by using the aspiration handle. The needle was moved forward and backwards in different directions to sample a wide area. Small drops of testicular aspirate usually accumulated in the transparent tube connected to the butterfly needle. The tube was then clamped and the needle withdrawn from the scrotum.

The needle was rinsed with HTF-HEPES medium supplemented with 0.4% human serum albumin (HSA, Sigma) into a four well plate (Nunc, Roskilde, Denmark). The aspirates were immediately examined for the presence of mature motile spermatozoa under an inverted microscope at ×200 magnification. According to our current practice, if spermatozoa were found in this initial aspirate evaluation,

testicular sampling was stopped. In all patients in this report, spermatozoa were not found at the initial evaluation and therefore ~10 punctures were performed in various locations in each testis.

Sperm preparation

Sections of seminiferous tubules in the testicular aspirate were minced in a Petri dish by means of two microscope slides or 21 gauge needles. All the testicular material was then pulled into a 15 ml conical tube and incubated at 37°C to allow the accumulation of debris. The supernatant above the debris was then transferred into another 15 ml conical tube and centrifuged at 1800 g for 5 min. The pellet was resuspended in 50 µl of HTF-HEPES medium supplemented with 0.4% HSA and transferred to the injection dishes. Each injection dish included five 2 µl drops of the sperm suspension, a central drop of 10% polyvinylpyrrolidone (PVP, Sigma) and three drops of medium for injection (HTF-HEPES with 6% SSS), all covered with pre-equilibrated paraffin oil (Sigma).

ICSI

The injection of spermatozoa was carried out according to the method of Van Steirteghem et al. (1993). The identification and injection of round spermatids were performed as previously described by Tesarik and Mendoza (1996). Both procedures were performed on the heated stage of a Nikon Diaphot inverted microscope (Nikon, Tokyo, Japan) equipped with two Leitz mechanical manipulators. Ready made injection pipettes (Swemede, Upsalla, Sweden) and holding pipettes (Cook) were used. The drops of sperm suspension were carefully screened under the inverted microscope for the presence of spermatozoa or spermatids. Round spermatids were identified according to their size (smaller than other spermatogenic cells, monocytes and polynuclear leukocytes) and the presence of a round centred nucleus surrounded by a regular zone of cytoplasm. Only when a sperm cell or a spermatid were located was an oocyte transferred from the incubator to one of the drops of medium for injection in the same injection dish. The injection pipette was then filled with PVP to collect the sperm cell or spermatid which were then rinsed in the PVP drop and injected into the oocyte. Spermatozoa were immobilized in the PVP drop before being injected. The round spermatids were slightly larger than the internal diameter of the injection pipette (7 µm) and therefore deformed upon aspiration. Cells that were deformed but did not disintegrate when transferred to the PVP drop before injection were assumed to be viable. The oocyte was washed with culture medium and placed for further incubation in HTF with 7.5% SSS for 16–18 h, after which fertilization was evaluated. Embryo morphology was scored before biopsy according to Staessen et al. (1992).

Embryo biopsy

Embryo biopsy was performed on the third day after ICSI in drops of a phosphate-buffered saline (PBS) without calcium and magnesium in the presence of 0.4% HSA and 0.1 M sucrose (Sigma) under pre-equilibrated paraffin oil. Biopsies were conducted by using the same microscope, manipulators and holding pipettes that were used for ICSI. A hole was drilled in the zona pellucida with a stream of acidified Tyrode's solution (pH = 2.4) that was expelled through a micropipette (internal diameter 8 µm). A single blastomere was sampled from each embryo through the hole in the zona pellucida, by gentle aspiration into a biopsy pipette (internal diameter 40 µm).

Interphase nuclei spreading

Each of the sampled blastomeres was first rinsed in a fresh droplet of the biopsy medium and then fixed individually in a small drop of spreading solution (0.01 N HCl, 0.1% Tween 20) on a poly-L-lysine coated slide according to Coonen et al. (1994). The slide was air dried and the location of the nucleus was marked by a diamond pencil. The slides were washed in PBS for 5 min and dehydrated through an ethanol series.

DNA probes for FISH

DNA probes for chromosomes Y, X and 18, which were directly labelled with blue, red and green (CEP® Spectrum aqua, orange and green; Vysis Naperville, IL, USA) fluorescent haptens respectively, were used. The hybridization targets for the 18 and X probes were α-satellite repeat clusters in the centromeric region of these chromosomes. The Y probe was specific for satellite III DNA at the heterochromatic region of the long arm of Y chromosome.

FISH

Our FISH protocol followed the guidelines of previous publications (Munné et al., 1993; Harper et al., 1994) with slight modifications. Slides were treated with pepsin (100 µg/ml) in 0.01 N HCl for 20 min at 37°C, rinsed in double distilled water followed by PBS and fixed for 10 min in 1% paraformaldehyde at 4°C. Slides were then rinsed in PBS and twice in double distilled water followed by dehydration through an ethanol series. The hybridization mixture included 1 µl of each of the directly labelled CEP® probes (40–50 ng/µl) and 7 µl of hybridization mix II solution (Vysis). It was applied to the slide under a 22×22mm coverslip and sealed with rubber cement. The slide was placed on a programmable slide warmer in a dark and moist chamber (HYBrite hybridization system; Vysis) for denaturation at 80°C for 3 min followed by hybridization at 37°C for 45 min. Post-hybridization washings included: 5 min in 50% formamide, 2×SSC, pH = 7.6, at 42°C; 5 min in 2×SSC, pH = 7.0, at 42°C; 5 min in 2×SSC, NP-40 0.1%, pH = 7, at 42°C and PBD (Na_2HPO_4 0.1 M, NaH_2PO_4 0.1 M, NP40 0.1%, pH = 8.0) at room temperature for 2 min. The slides were then counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in anti-fade solution. which was followed by signal analysis.

Fluorescence microscopy was performed with a Leitz Ploemopak microscope (Leica, Wetzlar, Germany). The nuclei which were counterstained with DAPI were located by using a single wave-length filter for DAPI (PSI Scientific Systems, Chester, England). The three probes were distinguished by using single band wave-length filters for orange and green (PSI Scientific Systems) and a triple band wave-length filter for aqua (Vysis). Fluorescent signals were analysed by using a computerized image capturing system (Powergene 760/121 Karyotyping, PSI Scientific Systems). The scoring criteria described by Hopman et al. (1988) were followed for fluorescent signals interpretation.

Results

Nine attempts at testicular sperm retrieval by FNA were performed in seven consecutive non-mosaic Klinefelter patients. In four patients, between one and five mature spermatozoa were retrieved per trial in five attempts (two consecutive attempts were performed in patient no. 2). Overall, three (19%) out of the 16 retrieved spermatozoa were motile. In another patient (patient no. 5), only 10 round spermatids were isolated from the testicular aspirates. In two patients, spermatozoa or spermatids were not isolated in three attempts (two consecutive attempts were performed in patient no. 6) (Table II).

Round spermatids were injected into ten MII oocytes, of which eight remained intact and four showed a single pro-nucleus 18 h after injection. One cleavage stage embryo

Table II. The outcome of testicular FNA, ICSI and PGD in five treatment cycles in which spermatozoa or spermatids were recovered

Patient no.	Cycle no.	No. spermatozoa		No. MII oocytes		No. zygotes			Embryos at 3 days			Biopsied blastomeres	FISH results		Embryo transfer
		Total	Motile	Injected	Intact	2PN	1PN	3PN	Embryo no.	No. cells	Morphology[a]		Biopsy	Whole embryo	
1	1	4	1	4	4	2			1	8	A	1	18,18,X,X.Y	Chaotic	No
									2	7	B	1	18,18,X,Y		Yes[b]
2	1	2	0	2	1	1			1	4	A	–			Yes
	2	4	0	4	2		2		1	8	A	–			Yes
									2	5	A	–			Yes
3	1	5	1	5	3	1			–						
4	1	1	1	1	1			1	–						
Total (%)		16	3 (19)	16	11 (69)	4 (36)	2	1							
5	1	10[c]		10	8		4		1	6	C	1	18,X,X	Chaotic	No

[a]Embryo morphology was scored before biopsy according to Staessen *et al.* (1992).
[b]A pregnancy and birth.
[c]Round spermatids.
FNA = fine needle aspiration; ICSI = intracytoplasmic sperm injection; PGD = preimplantation genetic diagnosis.

developed on day 3. A single blastomere was biopsied and analysed for chromosomes X, Y, and 18 ploidy by FISH. The result was 18,XX and therefore the embryo was not transferred. Whole embryo (five blastomeres) spreading revealed that only two additional blastomeres contained a nucleus; these were further analysed for chromosome X and Y ploidy. FISH results (YXX and YX) were consistent with a chaotic chromosome segregation pattern in a male embryo. These results confirmed that the embryo was not a result of oocyte activation.

Mature sperm cells were injected into a total of 16 metaphase-II oocytes. Eleven oocytes (69%) remained intact after the ICSI procedure. Normal fertilization, determined by the presence of two distinct pronuclei (2PN), was observed in four oocytes (36%). A single pronucleus (1PN) was documented in two oocytes and three pronuclei (3PN) were observed in an additional oocyte. Five cleavage stage embryos developed from the oocytes of two couples. Upon the request of one couple, their three embryos, of which two were derived from a 1PN fertilization, were transferred without PGD. Unfortunately pregnancy was not achieved. The second couple agreed that their two embryos, derived from normal fertilization, undergo PGD. Biopsy of a single blastomere was performed on each embryo, followed by single cell ploidy analysis of chromosomes X, Y and 18 by FISH. FISH results from one embryo were 18,18,X,X,Y (Figure 1) and according to the couple's request the embryo was not transferred. Analysis of the remaining blastomeres of this embryo, at a later stage, identified a variable number of chromosomes in the nuclei (18,X,Y in three nuclei, 18,18 in three nuclei, 18,Y,Y,X in one nucleus), consistent with chaotic chromosomal segregation. The second embryo, diagnosed as 18,18,X,Y (Figure 1) was transferred, resulting in a pregnancy and birth at term of a healthy male with a normal karyotype.

Discussion

The results of this study indicate that mature spermatozoa may be successfully retrieved from azoospermic non-mosaic Klinefelter patients by using testicular FNA. Following ICSI, these spermatozoa may induce normal fertilization, cleavage

and implantation, leading to the development of a pregnancy and birth.

In our series of seven consecutive azoospermic non-mosaic Klinefelter individuals, mature testicular spermatozoa were retrieved from four patients. This success rate is in line with the results of a recent report by Tournaye *et al.* (1996), in which testicular sperm cells were recovered from four out of nine individuals with 47,XXY karyotypes. In both series, three of the four individuals from whom spermatozoa were recovered had a history of presence of spermatozoa in their ejaculates as demonstrated either by light or electron microscopy. Nevertheless, more data from a larger series are still needed to determine the expected outcome of testicular sperm retrieval in non-mosaic Klinefelter patients and to judge the value of past demonstrations of spermatozoa in the ejaculate as a predictor of success.

In the report by Tournaye *et al.* (1996), testicular spermatozoa were retrieved by using an open multiple biopsy sampling method. Our results indicate that testicular FNA may serve as an alternative method to recover mature sperm cells in azoospermic non-mosaic Klinefelter cases. Percutaneous FNA has been considered to induce less trauma and postoperative morbidity compared to open multiple biopsies (Craft and Tsirigotis, 1995). Indeed, the procedure in our series was not associated with any postoperative complications and was highly acceptable to the patients, who could return to work the following day. It has also been suggested that the FNA technique may allow the sampling of more sites in the testis than the open multiple biopsy technique and therefore may have better prospects to sample a focal locus of spermatogenesis (Craft and Tsirigotis, 1995; Lewin *et al.*, 1996). However, in a single prospective study which compared both techniques among non-obstructive azoospermic individuals, testicular FNA had a significantly lower yield (Friedler *et al.*, 1997). Still, it seems that more comparative studies from additional centres are needed to conclude if any method should be preferred.

In this study, we have demonstrated that testicular spermatozoa recovered from azoospermic individuals with non-mosaic 47,XXY karyotype could induce normal fertilization and
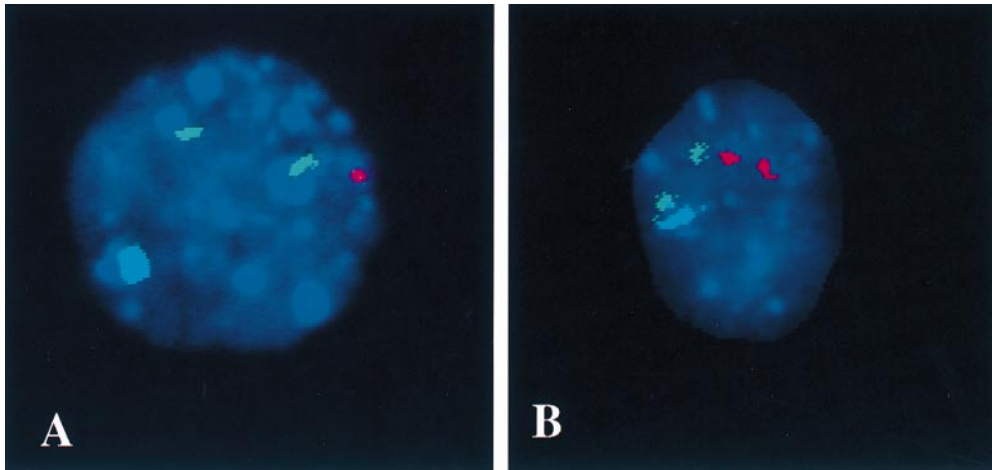
Figure 1. Triple FISH analysis of chromosomes X (red), Y (blue) and 18 (green) on the nuclei of single blastomeres. (**A**) A male nucleus with an X, Y and two 18 chromosome signals. (**B**) An abnormal nucleus showing two X, a single Y and two 18 chromosome signals.

cleavage following ICSI, as was previously reported by Staessen *et al.* (1996). The fertilization rate (37%) in our series was similar to the rate (42.1%) reported by Staessen *et al.* We further demonstrated that embryos which are derived from these patients can implant and produce a successful pregnancy and birth.

A non-mosaic 47,XXY karyotype was demonstrated in each of the individuals in our series by cytogenetic analysis of at least 30 peripheral blood lymphocyte metaphases. In five patients, an additional FISH analysis of 100–200 peripheral blood lymphocytes showed no evidence of mosaicism. However, it should be noted that our data cannot exclude mosaicism of the germ cell line.

The rate of sex chromosome aneuploidy among spermatozoa in individuals with a 47,XXY karyotype is unknown, as most of these patients are azoospermic. It has been assumed by some authors that only 46,XY germ cells would be able to complete meiosis (Luciani *et al.*, 1970; Laurent *et al.*, 1972). However, other investigators suggested, on the basis of meiotic observations of testicular biopsy material, that a few 47,XXY germ cells can initiate the meiotic process (Skakkebaek *et al.*, 1969; Vidal *et al.*, 1984). This view is supported by recent cytogenetic studies of sperm cells from individuals with Klinefelter's syndrome. Karyotyping (Cozzi *et al.*, 1994), as well as analysis by in-situ hybridization techniques (Chevret *et al.*, 1996; Martini *et al.*, 1996) of spermatozoa from mosaic individuals have demonstrated a significantly increased incidence (0.9–2.09%) of hyperhaploid 24,XY gametes. In addition, a recent unique FISH analysis of sperm cells from an oligozoospermic, apparently non-mosaic individual demonstrated an increase in the rate of both 24,XY (1.36%) as well as 24,XX (1.22%) gametes (Guttenbach *et al.*, 1997). These findings support the suggestion that some 47,XXY germ cells are able to go through meiosis and produce spermatozoa with gonosome hyperhaploidy. ICSI with such gametes would produce a progeny with sex chromosome aneuploidy.

Preimplantation FISH analysis of three embryos, in the present study, revealed a normal chromosome constitution in one embryo and a chaotic chromosomal segregation in the two other embryos. To the best of our knowledge, an embryo with a non-mosaic XXY or XXX chromosomal pattern has not been demonstrated to result from sperm cells which were obtained from individuals with 47,XXY karyotype (Staessen *et al.*, 1996, 1997; Hinney *et al.*, 1997). Nevertheless, the number of embryos evaluated so far is limited. Therefore, as the risk of gonosome aneuploidy among these embryos is unclear, and based on the above mentioned cytogenetic data of sperm cells, it seems justified at present to offer PGD and/or prenatal diagnosis to prove sex chromosome euploidy.

Whenever PGD is attempted, the patients should be consulted regarding the limitations of aneuploidy detection by this experimental technique. In some cases, blastomere analysis at the cleavage stage will not be representative of the whole embryo, due to the high frequency of chromosomal mosaicism in human embryos (Munné *et al.*, 1993, 1995; Harper *et al.*, 1995). Genetic misdiagnosis may also result from false FISH results due to signal overlapping or FISH failure. The consequences of these limitations were recently demonstrated by Munné *et al.* (1995), who reported an error rate of 5.4% for PGD by FISH of the common aneuploidies. Therefore, at present, PGD cannot substitute invasive prenatal diagnosis and fetal karyotyping should be strongly recommended.

In conclusion, the results of this report indicate that a pregnancy and birth may be attained in cases of non-mosaic Klinefelter's syndrome following testicular sperm retrieval by FNA combined with ICSI. These techniques may open new perspectives for fatherhood for these patients. Nevertheless, patients should be counselled regarding the experimental nature of this treatment. Due to the unknown risk of chromosomal imbalance in embryos from Klinefelter patients, PGD and/or prenatal diagnosis should be recommended.

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