Genetic risk of Klinefelter Syndrome in ART

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17 **Abstract**

18 Purpose: The main cause of Klinefelter Syndrome (KS) has been believed to be XY sperm.
19 Accordingly, in ICSI treatment of KS patients, hereditary KS has been a concern. Therefore,
20 we attempted to estimate the risk before and after the ART.
21 Methods: First, to validate the safety of KS patient's gametes, FISH analysis following an
22 original cell identification method using 1052 testicular gametes of 30 patients was
23 conducted. Second, in resultant 45 babies, cytogenetic and physical/cognitive screening data
24 was analyzed. In addition, we conducted a first attempt to investigate the origin of the extra
25 X in 11 KS using 12 X chromosome short tandem repeats (STRs) to estimate paternal
26 contribution on KS.
27 Results: No sex chromosomally abnormal gamete was found in FISH and the babies were
28 normal genetically, physical and cognitively. In STR, we confirmed that most (7/11) of KS
29 patients resulted from the fertilization of XX oocytes, suggesting that a KS baby previously
30 reported may not be resultant from XY sperm.
31 Conclusion: These results indicate that the risk of ART for KS patients is not as high as
32 previously expected.
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34 Key Words: Klinefelter Syndrome / Micro-TESE / ICSI / FISH / X-chromosome STR /
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Introduction

Klinefelter's syndrome (KS) is one of the most common chromosomal abnormalities found in newborns. Its incidence is approximately 1 in 500~1000 live birth males [1]. Almost all patients with KS have a non-mosaic 47, XXY karyotype [2] originally described as a syndrome with increased exertion follicle-stimulating hormone, gynecomastia, azoospermia [3, 4] and slightly low IQ. Once considered permanently infertile, these individuals can now reproduce using intracytoplasmic injection of spermatozoa extracted from their testicles [5]. To date, the birth of more than 200 babies has been reported around the world [6-23], 45 of those babies have been born at our institute, following ICSI with gametes of KS patients. Among those cases we know only one case of a fetus in a triplet pregnancy that was diagnosed with KS with amniocentesis, that case was later reduced to a healthy twin pregnancy [10]. On the other hand, there have been reports warning that the sex chromosome abnormality incidence increases in children born after ICSI from KS patients who produce sperm with XY disomy [24-29]. In contrast, other reports [12] showed that only normal sperm with haploid X or Y were found in KS patient's testis. Accordingly, it is important to collect more precise data to determine the risk level of ART using KS patient's gamete and decide how to deal with the clinical treatment of KS patients.

Recently, we established criteria to distinguish testicular somatic and meiotic cells without fixation and any staining [30], and accordingly precise cytogenetic analysis of these cells became possible. In this study, the criteria were used for a cytogenetic analysis (chromosome assay and FISH) of KS patient's sperm and meiotic cells selectively isolated, instead of the previous method with spermatogenic cell mixture. Furthermore, a follow-up review of comparably large number (N=45) of newborn babies born from the ART treatment of KS patients at our hospital from 2000 to 2013 was performed to confirm that XY sperm was not selected for the ART treatment. Namely, the risk of hereditary KS was examined before and after the ART treatment. In addition, we conducted a first attempt of X-chromosome short tandem repeats (STR) analysis among patients and their parents to estimate XY sperm and XX oocyte contribution to the birth of KS patients, since there is a possibility that KS resulted from XX oocyte fertilization may be more frequent.

Materials and methods

Patients

This study dealt with 280 men who had previously been diagnosed as having non-mosaic Klinefelter syndrome and had consented to receive Micro-TESE treatment at our institution from 2000 to 2013.

Biopsy of testis tissues

Several different sites of each testis were biopsied under operation microscope (Micro-TESE). Biopsied

testicular tissues were prepared in D-PBS containing 0.125% collagenase and 0.01% DNase to free spermatogenic cells from seminiferous tubules as previously described [30]. The cells were used for cytogenetic analysis or clinical treatments (ICSI or ROSI) after cryopreservation.

Freezing and thawing of sperm or spermatid

For cryopreservation of sperm, the testis tissue suspension was centrifuged and the pellets were transferred into a droplet of HTF on a petri dish with a glass pipette under a diverted microscope. Then, the cell suspension was mixed with a very small amount of a freezing medium (approximately 2 µl of HTF with 10% serum protein substances (SPS) and 100mM Sucrose), and placed on the tip of CRYOTOP under an inverted microscope. The Cryotop was exposed to liquid nitrogen vapor for 2 min and stored in liquid nitrogen. For thawing of the frozen cells, after maintaining the Cryotop in air for 5 seconds, it was dipped into a droplet covered with warm mineral oil (37˚C) to suspend the cells [31]. Motile sperm were selected and used for ICSI.

For ROSI, spermatids were selected from testicular cell suspension under an inverted microscope and suspended in 0.15 ml of freezing medium (D-PBS with 0.6 M ethylene glycol, 0.125 M sucrose and antibiotics). The suspension was drawn in a 0.25 ml Cassou straw and cooled on ice (4˚C). After the straw was maintained at -7˚C in a cooling chamber of a programmable alcohol-bath-freezer for 20 min, it was cooled to -30˚C at the rate of -0.3 ˚C /min before plunging into liquid nitrogen. Thawing was carried out by maintaining the straw in air for 5 seconds. The cell suspension was then diluted with HTF containing 10% SPS (SAGE In Vitro Fertilization; Cooper Surgical) in a test tube to remove cryoprotectant [30].

Cytological identification of testicular cells

We have already established criteria for identifying biopsied spermatogenic cells morphologically. Characteristics of testicular cells were examined in detail under a differential interference microscope (10 x 40), and then their chromosomal constitutions were determined by cytogenetic analysis to confirm whether the characteristics used and the meiotic phases correlate each other [30].

FISH procedure

Spermatogonia (SG), primary spermatocytes (Pr-Sc), round spermatids (ST) and elongating or mature sperm selected from enzymatically-treated biopsied tissues with a micromanipulator were put into a droplet of HTF with SPS, and then placed in a droplet of 10 µl of PBS (C^{2+} and Mg^{2+} free) on a Poly-L-Lysine coated slides with a square mark on the back slide. Soon after PBS surrounding cells was completely dried off, the cells were covered with small amount of the fixative of Carnoy's solution (methanol:acetic

acid=3:1). The fixative evaporated gradually and the cells became transparent. When the fixative completely disappeared, the cell membrane burst and nuclei were attached to the glass slide. In order to wash away phosphate crystals derived from PBS (-), the cells were covered with the fixative several times and dried them in air. For the fixed cells, triple target FISH was performed using fluorescence labelled DNA probes specific for chromosomes X, Y and chromosome 7 (Vysis, CEP DNA probe). Mixture of the probes was applied to the slide under a coverslip and the nuclear and probe DNA were denatured simultaneously for 5 min at 75 ˚C. The slide was then incubated in a chamber (Hybrite, Vysis) at 42 ˚C for 120 min to allow hybridization, and then counterstained with DAPI [32].

Chromosome assay of spermatogenic cells

For chromosome analysis of spermatogenic cells that were identified with our morphological criteria, chromosome assay with inter-specific injection into mouse MII oocytes was used [33]. The meiotic cells were injected into mouse oocytes 10 min after electrical stimulations (AC 8V/cm 1000 KHz for 8sec and DC 1200V /cm for 99μsec). Sperm or elongating sperm were injected without electric stimulations. After overnight incubation in HTF containing 50ng/ml Vinblastine, the nucleus of meiotic cells or sperm injected was allowed forming chromosomes in the mouse oocytes. Chromosome slides of the oocytes were prepared by the gradual fixation-air drying method [34].

Microinjection of testicular sperm or spermatid

ICSI was conducted with motile and morphologically normal sperm. Oocyte penetration by spermatid was conducted with a comparably larger injection pipette than that used for conventional ICSI. In the cases of Sa~Sb spermatid, oocyte activation with electrical stimulation was required before injection [30], no oocyte activation for Sc~Sd. After five days of incubation, the blastocysts were transferred into the uterus.

X-chromosome short tandem repeats (STR)

Using blood or oral mucous samples of 11 KS patients and one or both of their parents with their consent, the origin of the extra X chromosome was determined with X-chromosome haplotype markers (short tandem repeats of 12 loci), according to the method by Shrivastava et al. [35]. With DNA extracted from the samples, multiplexed PCR amplifications of the 12 X-STR loci (linkage group 1, DXS10148, DXS10135, DXS8378; linkage group 2, DXA10079, DXS10074, DXS7132; linkage group 3, HPRTB, DXS10101, DXS10103; linkage group 3, DXS10134, DXS10146, DXS10146) and AMELOGENIN were conducted using an Investigator Argus X-12 QS Kit (Quigen, Germany). Electrophoresis was run on an ABI PRISM 3100 Genetic Analyzer for the PCR products. The data obtained was analyzed with

GeneMapper ID software. All the steps described above were entrusted to Tohoku Chemical Co., Ltd. (Japan).

Screening of babies and children

Out of 45 babies who were born by ICSI or spermatid injection treatment of KS patients in our hospital, 29 underwent chromosomal analysis using amniocentesis or peripheral blood samples before or after birth, respectively, along with the compulsory newborn or infant screening for physical and cognitive development in Japan. For the rest, only information in the screening was used to examine the possibility of KS syndrome.

Ethics

Clinical application of Round spermatid injection (ROSI) and genetic analysis with X-chromosome short tandem repeats (STR) were approved by the Institutional Review Board of the Saint Mother Obstetrics and Gynecology Clinic. This clinical study was also registered on the University Hospital Medical Information Network of Japan (UMIN Clinical Trials Registry: UMIN000006117, UMIN000024542) adhering to the ICMJE criteria.

RESULTS

Morphological characteristics of spermatogenic cells

In Fig 1a, typical images of spermatogenic cells are shown. Elongating and elongated spermatids were easily identified with deviated condensed nuclei and short flagella, respectively. It was comparably difficult to distinguish among SG, early primary spermatocyte and round spermatids. Round spermatids were the smallest spermatogenic cells (6-8 μm in diameter; slightly smaller than erythrocytes). They were much smaller than spermatocytes (10-12 μm) and slightly smaller than SG (8 -10 μm). Two to three nucleoli were seen within nuclei of SG and spermatocytes, but not in round spermatids. Area of the cytoplasm surrounding nucleus was narrower in round spermatids than in spermatogonia. Protruded active pseudopodia were often seen in SG [36], but not in round spermatids. Although an acrosomal vesicle or cap was considered to be a strong evidence of the cell being a round spermatid, such structures were found in less than 10% of presumptive spermatids.

Chromosome abnormality in testicular cells of KS patients

In the Fig 1b and 1c, chromosome images of normal spermatogenic cells visualized by chromosome assay and FISH analysis are shown, respectively. When chromosomes of spermatogenic cells were induced to

condense in mouse oocytes, SG had 46 of dyad chromosomes, which are seen at the metaphase of somatic cell proliferation. Pr-Sc had 23 of tetrad chromosomes, in some of which the cross-overs were observed (see arrow in Fig 1b). ST had 23 monad chromosomes. Therefore, the chromosome assay showed that our criteria for spermatogenic cell morphology allowed them to be identified correctly, and accordingly we could apply the cells which were selected with the criteria for FISH analysis of their interphase nuclei. In SG, 2 blue spots of chromosome 18 and a green and orange spot of X and Y are found. In Pr-Sc each one spot of chr-18, X and Y were visible, 3 spots in total. In ST a blue spot of chr-18 and either of green (X) or orange (Y) spot, a total of 2 spots were observed (Fig 1c).

The results of FISH analysis for SG, Pr-Sc and ST in 5 KS men are shown in Table 1. The mean values of age, testicle volume, FSH, LH and Testosterone were 33.2 years old, 11.2 ml, 4.08 mIU/ml, 4.32mIU/ml and 4.48 ng/ml, respectively. In the SG stage, the average proportion of 46 XY and 47 XXY was approximately 73.6% (194/265) and 26.4% (71/265), respectively. In one case (#2), all SG was 46 XY of chromosome constitution (34/34). Until now there have been few reports describing the high percentage of 46 XY compared to 47 XXY. This result deserves attention. In contrast, in all 5 KS men, no sex chromosomally aberrant Pr-Sc was found (0/256) and resultant STs (467 cells) were also normal with haploid X or Y in almost equal proportion (49:51). This result seems to support strongly the probability that chromosomally normal sperm and STs are derived from meiosis of chromosomally normal Pr-Sc in KS patients. In addition, in other 25 KS patients, 100 sperm (n=10) or 485 ST (n=15) selected were subjected to FISH analysis to estimate the risk of accidentally selecting gametes with sex chromosome aneuploidy for ICSI. In conclusion, no sex chromosome abnormalities have been observed in the KS patient's gametes until now.

The success rate of ICSI/ROSI

The success rates of the ICSI/ROSI treatment of KS patients are shown in Table 2. Out of 280 patients, sperm with faint motility were recovered in 92 (32.9%) patients for ICSI. Spermatids, which are evidence of the completion of meiosis, were found in 33 (11.8%) patients for ROSI. In 155 (55.4%) patients, no spermatogenic cells were found. The incidences of pregnancy per treatment cycle, miscarriage and delivery were 12.4% (59/477), 37.3% (22/59), 7.8% (37/477), respectively. Finally, 45 healthy babies (including 6 twins and 1 triplets cases) were born (♂:♀= 21:24).

Physical and cognitive development of KS patient's babies

In the 29 babies who were cytogenetically analyzed, it was confirmed that they had a normal karyotype. The results of the newborn or infant screening for physical and cognitive development also showed that in

all 45 babies, no abnormality has been found.

Origin of extra X-chromosome in KS patients

Examples of X-chromosomal STR DNA profile are shown in Table 3. The KY patient number 09 is a case when both X-chromosomes were inherited from the mother (maternal origin). Allele of DXS10148 locus was 22.1 in the patient, 20 in his father and 20, 22.1 in his mother, suggesting that the patient's allele was inherited from maternal X-chromosomes. In other loci, all alleles of the patient were consistent with those of the mother. In the patient case of paternal origin (22TK), all alleles of 12 X-chromosome loci were inherited from the father, indicating that patient's X-chromosomes were send from both father and mother. In 63.6% (7) of the patients examined the X-chromosome was inherited from the mother and in 36.4% (4) of the 11 cases from the father. In the patients who had two maternal origin X-chromosomes, the cause of KS is that an extra X chromosome was left in an oocyte as a result of chromosomal non-disjunction at the 1st or 2nd meiotic division. In the patient who had X-chromosomes inherited from the parents, fertilization of XY-sperm is the cause of KS.

DISCUSSION

Genetic risk of the ICSI treatment for KS patients

The present study showed that 45 babies were successfully delivered using oocyte penetration by sperm or spermatid from KS patients from January 2000 to December 2013 at our hospital, and among them there was neither a case of chromosomal abnormality nor any case of physical or cognitive abnormality. The miscarriage rate (37.3%) in the treatment of KS patients using sperm and spermatid was not significantly higher when compared with non-KS patients (20.1% of 134) [30]. The results indicate the possibility that the genetic risk of the embryos produced in the treatment of KS patients is not as high as previously believed. This clinical result is consistent with the cytogenetic data of FISH and chromosome analysis in the gametes from KS patients. In 25 KS patients examined, no sex chromosome abnormality was found in 952 ST cells and 100 sperm (Table 2). The mechanism to produce normal gametes in KS patient's testis is considered to be as follows. In the patient #2, all SG and Pr-SC analyzed were XY in the sex chromosome constitution. Therefore, there is no doubt that ST with X or Y chromosome could be derived from meiosis of sex chromosomally normal germ cells. In the remaining 4 KS patients with testicular mosaicism of XY and XXY SG, it is difficult to determine which of XY or XXY cells were the source of ST. However, in all of their Pr-SC analyzed, sex chromosome constitution was XY, and accordingly all ST cells may have been produced from XY SG, suggesting the possibility that XXY SG cannot enter meiosis. Bergere et al. [12] have also reported that there was no XXY pachytene gamete and no increase of XY ST or XY sperm in 3

testicular 46 XY/47XXY mosaic KS patients, reading a conclusion that 46, XY cells can undergo meiosis. There is another possibility that the resultant abnormal daughter cells of XXY SG may become degenerative or apoptotic [37], because in this study only the spermatogenic cells that were alive with the intact plasma membrane and smooth round shape were selectively examined. This possibility seems to be a reason for an inconsistency of the present data with those of previous cytogenetic studies in KS patients. Many previous FISH studies have reported that not only sex chromosome abnormality rate but also the rate of autosomal aneuploidies [24] are higher in sperm from KS patients than from non-KS infertile patients [24-29]. In those studies, testicular cell suspension was directly smeared on a glass slide, treated with DTT and hybridized with FISH probes. Since after the successive treatment, the artificially swollen sperm heads were not allowed to evaluate morphology, a tail was used to identify sperm. Therefore, it cannot be denied that aberrant sperm heads, which are not appropriate for ICSI treatment, must have been analyzed along with normal sperm heads in the previous FISH studies. It is a clear fact that the risk of disomy and diploidy is higher in sperm with aberrant heads [38, 39]. In addition, this assumption is also supported by the high frequency of XY sperm found in the control donor sperm used in the FISH studies because the rates of XY sperm obtained were 20 to 100 times higher than the rate (0.018%) reported by Kamiguchi et al. [40] using chromosome assay of 15,864 ejaculated donor sperm (n=51) which penetrated hamster oocytes. We understand that the reason of the distinct results between our and previous FISH studies cannot not be revealed without a comparative study among the different sperm selection procedures. However, it can be concluded that instead of using testicular cell suspension, our cytogenetic studies with spermatogenic cells that were morphologically evaluated and selected is more suitable for exact estimation of the genetic risk in the ICSI treatment of the KS patients. On the other hand, Coates et al. [41] reported an increase of sex chromosome aneuploidy in array comparative genomic hybridization with the trophoblasts biopsied from embryos that were obtained by ICSI treatment of oligozoospermia males, suggesting the risk of the use of suboptimal sperm. Although it is not clear whether KS patients are included in their data, the result seems to disagree with the present data. However, their data includes some points that are hard to understand. First, the total aneuploidy rates did not differ among the embryos of IVF, ICSI with normal and suboptimal sperm groups. Second, in embryos of the suboptimal sperm group, aneuploidy increased in specific autosomes in addition to the sex chromosomes. These incompatible phenomena seem to be explained by the possibility that patients with genetic backgrounds causing aneuploidy of a specific chromosome(s) are contained in the oligozoospermia group. Therefore, their result may not be necessarily applicable to KS patients, although we have to pay close attention to the genetic risk of ICSI treatment of KS patients.

Contribution of XX oocyte in production of KS syndrome

When we found that no XY aneuploidy was observed in KS patient's gametes in our cytogenetic analysis, we hypothesized that XY sperm did not contribute to the production of KS syndrome as much as XX oocytes. In our X-chromosome STR analysis, the patients with maternal origin X-chromosomes were comparably frequent (63.6%), suggesting that contribution of XX oocyte to the production of XXY embryos may be greater than XY sperm, although the sample number applied for X-chromosomal STR DNA profiling is not large enough. Some studies have previously attempted to determine the origin of the extra-X chromosome in KS patients with X chromosome restriction site polymorphism [42-44]. Maternal contribution to the production of KS syndrome was slightly greater in two studies (59% versus 41%) and was slightly lower in one study (42.8 % versus 57.1%). In those studies, however, there were cases that X chromosome origin was determined by appearance or disappearance of a single band in a single allele, which may have resulted from mutation. In X-chromosome STR analysis, the 12 X-chromosomal markers are clustered into 4 linkage groups, which consist of 3 alleles, and thus each set of three markers is handled as a haplotype for genotyping to avoid misjudgment. We could find no report that applied X-chromosome STR with PCR to KS patients in previous studies. Maiburg et al [45] reported that the extra X chromosomes is the result of meiotic nondisjunction or possibly, as recently described, of premature separation of sister chromatids both in paternally or maternally because of an increased maternal age [1, 46]. Since X-chromosome origin may affect the potency of spermatogenesis in KS patients, we will collect further data using this method.

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Disclosures

Conflict of interest: Tamito Miki, Motoi Nagayoshi, Yoichi Takemoto, Satoru Takeda, Seiji Watanabe, Atsushi Tanaka declare that they have no conflict of interest.

Human rights statements and informed consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients for being included in the study.

Animal studies: All institutional and national guidelines for the care and use of laboratory animals were followed.

Approval by Ethics Committee: This study was approved by the Institutional Review Board of the Saint Mother Obstetrics and Gynecology Clinic.

Clinical Trial Registry: University Hospital Medical Information Network of Japan (UMIN Clinical Trials Registry: UMIN000006117 and UMIN000024542)

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Figure legend

Fig 1. Morphology and chromosomal constitution of a normal spermatogonium, primary spermatocyte or round spermatid.

The three types of spermatogenic cells identified under a differential interference microscope (a), their chromosome complements formed in mouse oocytes (b), and fluorescent signals of X, Y and 18 chromosomes by FISH analysis (c).

Figure 1. Chromosomal analysis and FISH results in nuclei of spermatogenic cells

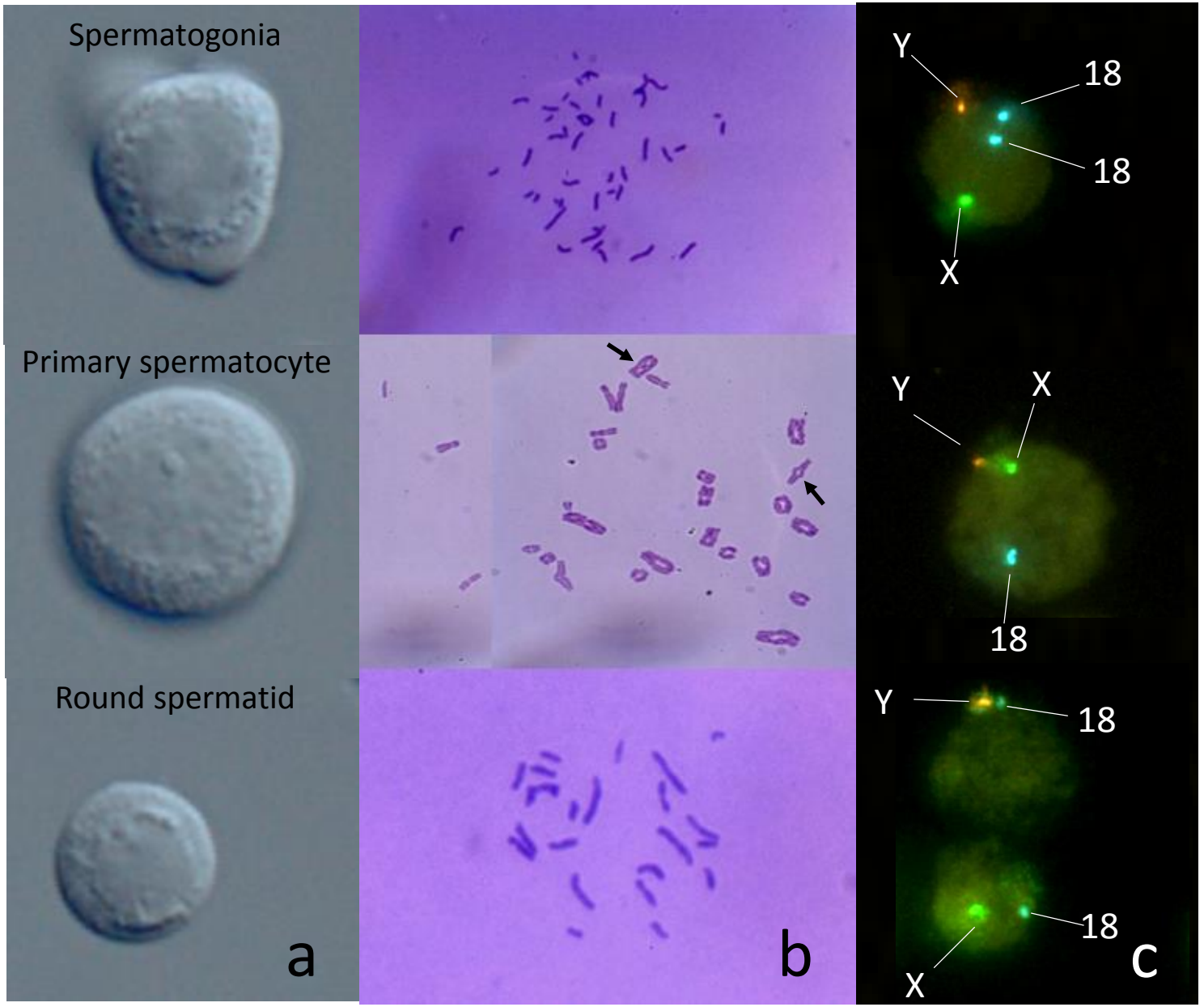

Table 1. FISH analysis of spermatogenic cells from 5 KS patients

Patient	Spermatogonia		Primary spermatocyte		Spermatid		
	XY (%)	XXY (%)	XY (%)	XXY (%)	X (%)	Y (%)	Other (%)
1	79 (95.2)	4 (4.8)	106 (100)	0	132 (49.8)	133 (50.2)	0
2	34 (100)	0 (0)	31 (100)	0	15 (41.7)	21 (58.3)	0
3	21 (45.7)	25 (54.3)	36 (100)	0	24 (48.0)	26 (52.0)	0
4	33 (57.9)	24 (42)	49 (100)	0	27 (49.1)	28 (50.9)	0
5	27 (60.0)	18 (40.0)	34 (100)	0	31 (50.8)	30 (49.2)	0
Total	194 (73.6)	71 (26.4)	256 (100)	0	229 (49.0)	238 (51.0)	0

Table 2. Clinical outcome of micro-fertilization using KS patient's gamete

	Sperm	Spermatid (Sa, Sb)	Spermatid (Sc, Sd)
No. of patients	92 (32.9%)	8 (2.9%)	25 (8.9%)
Age of wife (yrs)	31.2 (25-36)	30.5 (24-34)	29.5 (23-33)
No. of collected oocytes /patient	12.4 (8-20)	11.4 (7-19)	10.4 (6-15)
No. of fertilized oocytes /patient	8.1 (6-15)	7.9 (5-13)	7.5 (5-11))
% of good Day 3 embryos	60.4% (4.9/8.1)	30.3% (2.4/7.9)	44.0% (3.3/7.5)
Implantation rate*	17.4% (51/293)	14.5 (9/62)	16.4 (20/122)
Pregnancy rate*	13.7% (40/293)	9.7% (6/62)	10.7% (13/122)
Miscarriage rate*	32.5% (13/40)	66.7% (4/6)	38.5% (5/13)
Delivery rate*	9.2% (27/293)	3.2% (2/62)	6.6% (8/122)

* per ET cycles

45 babies were delivered in 37 cases that include 6 twins and 1 triplet pregnancies.

Table 3. Examples of X-chromosome STR profiles of KS patients and their parents.

Maternal origin of extra X-chromosome				
Patient #	**Markers**	**Father**	**Patient**	**Mother**
09KY	**DXS10148**	*20*	**22.1**	20, **22.1**
	DXS10135	*22*	**22**	21, **22**
	DXS8378	*10*	**10**	**10**
	DXS10079	*18, 21*	**18**, **20**	17, **18**, **20**
	DXS10074	*17*	**18**	16, **18**
	DXS7132	*16*	**14**	**14**, 15
	HPRTB	*12*	**14**	13, **14**
	DXS10101	*31.2*	**29**, **31.2**	**29**, **31.2**
	DXS10103	*18*	**17**, **19**	**17**, **19**
	DXS10134	*35*	**36**, **37.3**	**36**, **37.3**
	DXS10146	*24, 40.2*	**26**, **32**	**26**, **32**
	DXS7423	*14*	**15**, **16**	**15**, **16**
	AM	*X, Y*	X, *Y*	X
Paternal origin of extra X-chromosome				
22TK	**DXS10148**	*25.1*	**24.1**, *25.1*, **28.1**	24.1, 27.1, **28.1**
	DXS10135	*26*	**19**, *26*	**19**, 31
	DXS8378	*12*	**11**, *12*	**11**
	DXS10079	*16*	**15**, *16*, **21**	**15**, 19, **21**
	DXS10074	*18*	**17**, *18*	**17**, 19
	DXS7132	*14*	***14***	13, **14**
	HPRTB	*13*	**12**, *13*, **14**	**12**, **13**, **14**
	DXS10101	*31*	*31*, **31.2**	**31.2**, 32.2
	DXS10103	*19*	**18**, *19*	**18**
	DXS10134	*34*	*34*, **36**	**36**
	DXS10146	*28*	**26**, *28*	**26**
	DXS7423	*14*	*14*, **16**	15, **16**
	AM	*X, Y*	X, *Y*	X

Italic: paternal allele, **Bold**: maternal allele, Underlined: allele may be inherited from the parents