Spermatogenesis in Klinefelter syndrome

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ABSTRACT. Background: Klinefelter syndrome (KS) (47,XXY) is the most common sex chromosomal disorder, and it is a frequent form of male hypogonadism and infertility. Although the majority of these patients are azoospermic, they might have severe oligozoospermia or residual single-residual foci with spermatogenesis in the testis. Aim: We report our experience on sperm retrieval in the ejaculate and testis, and evaluate the frequency of chromosome abnormalities in sperm of KS. Subjects and methods: Eighty-four 47,XXY KS were evaluated with seminal analysis, body hair distribution, reproductive hormones, ultrasonographic scanning of the testis and prostate, bilateral testicular sperm extraction (TESE), sperm or testicular cells sex chromosomes aneuploidies. Results: Out of 84 patients, 7 (7/84; 8.3%) had sperm in the ejaculate. Out of the 77 azoospermic patients, 24 underwent TESE and 9 (9/24; 37.5%) had successful sperm recovery. The comparison of reproductive hormones, age and testicular volume did not show significant differences between patients with and without successful sperm recovery in semen or TESE. Patients without successful sperm recovery in semen analysis or TESE had signs of hyypoandrogenism more evident than patients with successful sperm recovery. Patients with KS produced a higher number of sperm aneuploidy with respect to normozoospermic fertile controls and non-genetic severely oligozoospermic men. Conclusions: Men with KS are not always sterile. In some of these patients sperm can be found in semen or in the testis, but the proportion of sperm aneuploidy is high. Signs of hypoandrogenism seem to be associated with low sperm recovery rate.


INTRODUCTION

Klinefelter syndrome (KS) (47,XXY) is the most common sex chromosomal disorder, with a prevalence of 1:600 and it is a frequent form of male hypogonadism and infertility (1, 2). KS is characterized by the presence of one or more extra X chromosomes; about 80% of cases have 47,XXY karyotype, the remaining 20% have higher-grade chromosome aneuploidies (48,XXX, 48,XXXY, 49,XXXXY), mosaicsisms (46,XX/ 47,XXX) or structurally abnormal X chromosomes (1). The chromosome aberration arises by maternal or paternal non-disjunction during meiotic division in germ-cell development, or rarely in early embryonic mitotic cell divisions (1).

The clinical features of KS commonly include hypogonadotropic hypogonadism, gynecomastia, small testes and azoospermia (3), but KS may be associated also with an increased risk of systemic diseases including malignancies, osteoporosis (4), venous thromboembolism (5), diabetes and cardiovascular diseases (6-8).

Usually these patients are azoospermic and their seminiferous tubules appear fibrotic and hyalinized (3, 9, 10) probably caused by the extra X chromosome, although some studies have reported the presence of mild oligozoospermia or criptozoospermia in subjects with the classical KS (1, 11, 12). Furthermore, it has been demonstrated that also azoospermic patients may present single-residual foci with spermatogenesis (13). Spermatogenesis in KS might be explained by two mechanisms: testicular mosaicism with 46,XY spermatogonia able to normally differentiate and spermatogenesis arising from 47,XXY spermatogonia. In fact, we have previously demonstrated that 47,XXY spermatogonia are capable of completing the spermatogenetic process leading to the formation of mature spermatozoa (13). Consistent with the hypothesis that 47,XXY germ cells may undergo and complete meiosis, aneuploidy rate for XX- and XY-disomies in ejaculated sperm is also increased with respect to controls, whereas the percentage of YY-disomies is normal (14). Although the great majority of children born by intracytoplasmic sperm injection (ICSI) from Klinefelter subjects are chromosomally normal, the risk of producing offspring with chromosome aneuploidies or higher risk of abortion rate is significant.

On the basis of these data several studies have demonstrated successful pregnancies in patients with no sperm in the ejaculate using testicular sperm extraction (TESE) and ICSI techniques. Success of sperm recovery with TESE was reported in 16 to 69% of cases (15-21) and several clinical parameters such as testicular volume, patient’s age, serum FSH, LH, free and total testosterone, presence of gynecomastia, were assessed for the predictive value for sperm recovery (18, 20-24). However, these studies reported contradictory results and no parameter has been accepted as predictive of successful sperm retrieval. This study, in men with non-mosaic KS, reports our experience on successful sperm retrieval in the ejaculate and by TESE, evaluating also the frequency of chromosome abnormalities in sperm. Furthermore, we have investigated the presence of possible predictive factors of successful sperm recovery.
MATERIAL AND METHODS

Patients and clinical analysis

We studied, in a retrospective study, a total of 84 patients having non-mosaic KS (mean age 30.9±8.4 yr; range 15-58 yr), diagnosed in the Center for Male Gamete Cryopreservation at the University of Padua from October 2005 to December 2008. The study was approved by the Hospital Ethics Committee and each participant gave his written informed consent.

All patients underwent peripheral karyotype analysis, evaluating at least 50 peripheral blood lymphocyte metaphases. Each man had never received testosterone substitution at the time of evaluation and was studied with semen analysis, measurement of concentrations of reproductive hormones, hemochromome, prostate-specific antigen (PSA) and ultrasonographic scanning of the testis to evaluate testicular morphology and volume and of the prostate to evaluate size. Body hair was scored in 13 body regions (upper lip, chin, cheek, lower and upper abdomen, pubic area, neck, axilla, chest, lower and upper back, arms and legs) using a semiquantitative method, based on a modification of the criteria of Ferriman and Gallwey (25) and Tanner (26) defining the total body hair score as the sum of all body regions. Densities were scored from 0 (no hair) to 4 points (maximal hair development) for each region. All patients with sperms in semen analysis were analyzed for numerical alterations of sperms sex chromosomes by means of fluorescence in situ hybridization (FISH). In 5 azoospermic patients FISH was applied on testicular cells retrieved by fine needle aspiration. Twenty-four azoospermic patients underwent bilateral TESE to retrieve sperm.

Semen samples were obtained by masturbation; after liquefaction at room temperature, semen volume, pH, sperm concentration, motility and morphology were determined following World Health Organization guidelines for semen analysis (27). Azoospermia was confirmed after 2 to 3 semen analyses and an examination of the pellet suspension subsequent to centrifugation at 1500 g.

Hormone assays

Blood was collected in the fasting state between 08:00 and 10:00 h. Serum FSH, LH, total testosterone and estradiol were evaluated by commercial electrochemiluminescence immunoassay methods (Elecsys 2010, Roche Diagnostics, Mannheim, Germany). For all parameters the intra- and interplate coefficients of variation were <8% and 10%, respectively. Free testosterone was calculated from total testosterone, total sex hormone-binding globulin (SHBG) concentrations, and the method of Vermeulen (28).

Inhibin plasma concentrations were measured by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) specific for the dimeric inhibin-B form (Serotec, Oxford, UK). Assay sensitivity was 15 pg/ml and the intra- and interassay coefficients of variation were 6.4 and 6.8%, respectively.

FISH

Numerical chromosomes alterations of sperm cells and of testicular cells retrieved by needle aspiration were evaluated by multicolor FISH as previously reported (13, 14). Briefly, cellular nuclei were decondensed according to the method proposed by Martini et al. (29). After decondensation, slides were immediately used for the successive steps or were stored in a refrigerator (2-4 days, 4 °C). DNA hybridization was performed using human α-satellite probes specific for chromosomes X, Y, and 18 (Amersham Life Sciences, Milan, Italy) directly labeled using fluorochromes FluorX (chromosome X, green) and Cy3 (chromosome Y, orange): for the detection of chromosome 18, a mixture (1:1) of FluorX and Cy3 directly labeled specific probes was used, resulting in a yellow signal. DNA denaturation of cells and probes, incubation, and posthybridization washing were performed following the Amersham protocol. Cellular nuclei were successively counterstained (1 min at room temperature) in a Coplin jar containing a phosphate-buffered saline (pH 7.4)-49,6-diamidino-2-phenylindole dihydrochloride solution (20 ng/ml). Slides were then rinsed in distilled water, air-dried in the dark, mounted using an antiadhesive solution [glycerol-distilled water, 9:1-1,4 diazabicyclo-(2-2,2)octane, 2%, wt/vol], and stored (1-4 days, 4 °C) or immediately observed using a Leica Diaplan epifluorescence microscope (Leica, Wetzlar, Germany) fitted with a 100-watt mercury lamp and a triple bandpass filter suitable for the fluorochromes in use. This procedure allows the detection of all probes as bright, compact, and uniformly sized spots. Each spot was evaluated and scored as specific for the chromosome corresponding to its color only when the intensity and size were similar to those of spots of the same color in the surrounding cells. Furthermore, if two spots of the same color were located in the same cell, the distance had to be more than their diameter for them to be considered distinct chromosomes (30). For each patient, at least 1000 sperm in the ejaculate or at least 100 spermatogenic cells in the tests have been scored. DNA probes were provided by Amersham Life Sciences (Milan, Italy). 49,6-Diamidine-2-phenylindole dihydrochloride was purchased from Boehringer Mannheim (Milan, Italy). All other chemicals were purchased from Sigma Chemical Co. (Milan, Italy). Patients affected by KS were compared with 108 normozoospermic fertile control men and 387 idiopathic severely oligozoospermic men, without constitutional genetic alterations, who presented a sperm concentration comparable to Klinefelter patients.

Testicular sperm extraction

TESE was performed under sedation with spermatic cord block with 0.5 % bupivacaine solution. After transverse incision of the tunica albuginea on the anterior surface of the testis, two small specimens of a diameter of approximately 5 mm were excised from each testis utilizing sharp scissors. Testis specimens were placed in a Petri dish with 2 ml of Biggers, Whitten and Whittingham (BWW) medium (Irvine Scientific, Santa Ana, CA, USA), mechanically sectioned by means of sterile slides and then further gently minced by sterile needles. After testicular shredding the testicular suspension was vortexed for 5 min and then transferred into a 15-ml conical tube containing 2-ml fresh medium and centrifuged at 1200 x g for 10 min. The supernatant was then discarded, the pellet resuspended with 1 ml of BWW medium and vortexed for 2 min. Under an inverted microscope at × 400 magnification, the testicular suspension was checked for the presence of mature sperm. If sperm were found, the sample was frozen with the method described previously (31) for future ICSI use.

Statistical analysis

Data in tables are presented as mean±SD of the mean. Differences between groups were evaluated by unpaired two-sided Student’s t-test. Comparisons of means of aneuploidy rates among groups were analyzed by Wilcoxon rank sum test. P values <0.05 were considered as statistically significant.