Sperm ultrastructure and 18, X, Y aneuploidies in a man with a 46 XY, 47 XY+18 mosaic karyotype: case report

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Abstract

Purpose To describe sperm morphology and meiotic segregation in the case of a man with a 46 XY/ 47 XY+18 mosaic karyotype.

Materials and methods A 25-year-old man came to our Centre for semen analysis. Morphological sperm evaluation was performed by light and electron microscopy; meiotic segregation was examined by fluorescence in situ hybridisation (FISH) technique using probes for chromosomes 18, X and Y. PCR was carried out on DNA from peripheral blood lymphocytes to analyze Y microdeletions.

Results Mathematically elaborated transmission electron microscopy data highlighted a low number of sperm devoid of ultrastructural defects, and the presence of characteristics of apoptosis and immaturity. FISH showed the presence of aneuploidies of chromosome 18 and sex chromosomes.

Conclusions In this case of mosaicism morphological and meiotic spermatogenetic impairment is shown, as well as structural chromosomal alterations.

Keywords Electron microscopy · FISH · Mosaic karyotype · Spermatozoa

Introduction

Trisomy 18 (Edwards's syndrome) is the second most common trisomy in man. It occurs in approximately 1:3,000 live births [1]. Since the first description of the syndrome by Edwards et al. [2], many aspects have been reported in the literature, including birth prevalence, phenotypes, parental age, seasonal variation and clustering with other syndromes [3].

In this paper we describe for the first time the case of a man showing a 46 XY/ 47 XY+18 karyotype. This condition, in which tissues of genetically different types occur in the same organism, is defined as mosaicism; the most common mosaic karyotypes are 45,X/46,XX and 45,X/46,XY. Another frequent mosaicism is 46,XY/47,XXY in which patients can have normal-sized testes [4], whereas this feature is absent in classic Klinefelter syndrome.

Some authors have reported that males with mosaic sex chromosomal abnormalities have no higher risk of producing offspring with a sex chromosomal abnormality by Intracytoplasmic Sperm Injection (ICSI) than OligoAstenoTeratozoospermic (OAT) males with a normal karyotype [5].

However, fluorescent in situ hybridization (FISH) studies in spermatozoa from carriers of sex chromosome mosaicism have revealed a significantly high number of hyperaploid spermatozoa [6, 7] and these patients may risk producing offspring with an altered sex constitution.

In this study we analysed spermatozoa from a 46 XY/ 47 XY+18 karyotype carrier. Sperm morphology was analysed by transmission electron microscopy (TEM), which provides a more detailed evaluation of sperm alterations than that of light microscope. FISH analysis was performed to detect sperm aneuploidies and a possible interchromosomal

We describe sperm morphology and meiotic segregation in a carrier of 46 XY/ 47 XY+18 mosaic karyotype.

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Table 1 Means and standard deviation of seminal parameters of two different samples from a man with 46 XY/47 XY+18 karyotype

Spermogram data		
Volume	Number sperm/ml $\times 10^6$	Motility (a+b)%
5.2 $\pm$ 1.414	7.175 $\pm$ 1.132	56 $\pm$ 5.657

effect (ICE). The combination of these two techniques could better clarify the spermatogenic process.

Case report

A-25-year-old man was referred to our Centre for semen analysis after one year of unprotected sexual intercourse without conception. His wife, aged 29, did not have any fertility problems. The patient provided written informed consent before inclusion in this study.

Sexual development, medical history and physical examination were normal. Microbiological investigations did not reveal any urogenital infection. The patient had never received hormone therapy.

Lymphocyte karyotyping performed with conventional Giemsa-Trypsin-Giemsa (GTG)-banding revealed that the patient showed a 46, XY/ 47, XY+18 karyotype. Two hundred metaphases were analysed. The normal karyotype was found in 92% of analysed metaphases, but in 8% the karyotype was 47, XY+18. For this reason, we analysed the chromosomal constitution of his parents and his brother and all showed a normal karyotype.

The hormonal pattern revealed a normal level of testosterone, FSH, LH and inhibin-B.

Semen samples were collected by masturbation after 4 days of sexual abstinence and examined after liquefaction for 30 min at 37°C. Volume, pH, concentration and motility were evaluated according to WHO parameters [8]. Semen analysis was repeated in two samples obtained during four months (Table 1). Sperm concentration was strongly reduced, whereas the total progressive motility, rapid (a) and slow (b), was normal in both samples. The eosin Y test detected 80–70% viable spermatozoa. Due to the reduced sperm concentration, PCR (Perkin Elmer Corp., Norwalk,

CT) screening of specific Y chromosome according to EAA (European Academy of Andrology) guidelines was performed and did not show any deletions in the investigated regions.

Sperm morphology was investigated by TEM (TEM; Philips Scientifics, Eindhoven, The Netherlands). Three hundred sperm were analysed in ultra-thin sections. TEM data was evaluated using the mathematical statistical formula of Baccetti et al. [9] which determines that the lower number of spermatozoa free of defects (“healthy”), assuring a normal fertility, is two million. Moreover, the mathematical tool is able to furnish the percentages of three main phenotypic sperm pathologies: immaturity, necrosis and apoptosis [10]. This method is only used by our laboratory, but it is validated by the large amount of paper published in the last 10 years. A software to elaborate TEM data has been developed and it was used by other groups, who collaborated with us [11].

In the analysed case the number of “healthy” sperm was lower (240.1 $\pm$ 103.078) (see Table 2) than 2 million, the minimum number of well structured sperm required to be considered fertile, and the main alterations were related to apoptosis and immaturity. Marginated chromatin, and swollen and badly assembled mitochondria were the typical ultrastructural markers of apoptosis (Fig. 1), whereas altered and reduced acrosome, a round nucleus with uncondensed chromatin and the presence of cytoplasmic droplets were the characteristic features of immaturity (Fig. 2).

Meiotic segregation was investigated by triple colour FISH for chromosomes 18, X, and Y probes (CEP, Chromosome Enumeration Probes, Vysis, IL, USA) carried out on the sperm nuclei according to Baccetti et al. [12] to evaluate aneuploidy frequency. Observation and scoring were performed using a Leitz Aristoplan Optical Microscope (Rockleigh, NJ) equipped with a fluorescence apparatus, with a triple bandpass filter for aqua, orange and green fluorochromes (Vysis, IL, USA) and a monochrome filter for DAPI. A total number of 7,368 (3,569 in the first sample + 3,809 in the second sample) sperm nuclei was scored.

The mean of frequencies of aneuploidy of chromosomes 18, X and Y are summarized in Table 3. In both examined

Table 2 Means and standard deviation of TEM spermatological characteristics in a man with 46 XY/47 XY+18 karyotype

Semen analysis	Semen profile	Number of healthy sperm	Percent sperm pathologies		
			Apoptosis	Immaturity	Necrosis
Patient	OT	240.1 $\pm$ 103.078	10.114 $\pm$ 0.167	79.172 $\pm$ 1.812	21.582 $\pm$ 1.689
Controls		>2 $\times$ 106	4.80 $\pm$ 3.40	55.10 $\pm$ 10.74	21.00 $\pm$ 14.94

O oligozoospermia; T teratozoospermia.

Fig. 1 TEM micrograph of longitudinal section of an apoptotic sperm characterized by margined chromatin (mCh) and altered acrosome (aA). A large cytoplasmic residue (CR) embeds the axoneme (AX) and disorganized, swollen mitochondria (M). X 13,500

samples, the mean of frequencies of diploidy, sex chromosome disomy, and particularly of chromosome 18 disomy, was higher than those in the control group.

Discussion

In the present study, we report seminal features and the meiotic segregation pattern in a patient, who had a rare mosaic 46 XY/47 XY+18 karyotype. To our knowledge, this is the first time that ultrastructural sperm analysis and FISH have been performed in a carrier of this type of mosaicism.

Recently, a correlation between abnormal karyotypes and human male infertility has become more evident [6, 7, 13–15].

In the analysed case a strongly reduced sperm concentration, despite normal progressive motility, was observed. For this reason, a screening of Y chromosome microdeletion was carried out but no Y microdeletions were detected.

Sperm morphology was evaluated by TEM analysis and the data were mathematically elaborated by the method used in our laboratory. Generally morphological sperm quality is performed by light microscopy, sometimes applying Kruger's criteria, but subcellular sperm anomalies

can only be detected by electron microscopy. TEM analysis was pivotal for clarifying the kind of anomalies present in the spermatozoa showing a normal motility. The impaired spermatogenic process was related to the presence of apoptosis and immaturity, pathologies already observed in spermatozoa from patients with different karyotype anomalies [14, 15].

FISH analysis on sperm nuclei highlighted an increased frequency of 18 chromosomes disomy and diploidy, whereas the frequency of sex chromosome disomy was slightly higher than normal value.

Meiotic behaviour of 18 chromosome could be related to the altered chromosomal constitution, the high frequency of sperm diploidies indicates incomplete meiosis process leading to immature sperm cells. It is not known whether sperm with abnormal morphology are the same as those considered containing aneuploidy nuclei; only the use of an electronic microstage locator allows to demonstrate that normal morphology is not an absolute indicator for the selection of genetically normal sperm [16].

The observed ultrastructural and FISH features suggest a spermatogenic derangement probably due to altered karyotype, but a poor testicular function cannot be excluded.

Morphological and meiotic spermatogenic impairment detected in the patient carrier of mosaicism make this man a potential candidate for preimplantation genetic diagnosis (PGD) for aneuploidy. This approach is aimed to provides

Fig. 2 TEM micrograph of longitudinal and cross sections of sperm cells. An immature sperm is characterized by a round nucleus with uncondensed chromatin (uCh) and a reduced acrosome far from the nucleus (arrow). A large cytoplasmic residue (CR) embeds the round, immature head. Cross sections at the middle piece level (MP) of the tails, highlight regular axonemal patterns and mitochondria assembly. A sperm with a normal nucleus (N) and with an acrosome presenting a loop is shown. X 17,000

Table 3 Frequencies of disomies and diploidies of chromosomes 18, X and Y in a man with 46,XY/47XY+18 karyotype

Semen analysis	Number of cells scored	Percent diploidy	Percent chromosome 18 disomy	Percent sex chromosome disomy
1	3,569	0.308	0.364	0.282
2	3,809	0.366	0.314	0.287
Fertile controls		0.280±0.006	0.110±0.003	0.230±0.004

information for embryonic chromosomal assessment, improving the chances of healthy implantation and increasing the efficiency of assisted reproductive techniques.

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