Structural sperm and aneuploidies studies in a case of spermatogenesis recovery after the use of androgenic anabolic steroids

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Abstract *Purpose:* Azoospermia may sometimes be related to the use of androgenic anabolic steroids. We report the case of an azoospermic man who had abused androgenic anabolic steroids and who recovered spermatogenesis six months after cessation of abuse and the administration of hormonal therapy. *Methods:* An azoospermic 34-year-old man came to Regional Referral Center for Male Infertility. The recovery of spermatogenesis was observed after the cessation of abuse of steroids and the administration of hormonal therapy. Ultrastructural analysis of sperm was carried out by transmission electron microscopy, and the meiotic segregation of chromosomes 1, 9, 18, X, Y was investigated. *Results:* Mathematically elaborated transmission electron microscopy data highlighted seminal features close to normal fertility. Fluorescence *in situ* hybridisation showed a high frequency of XY disomy in sperm. *Conclusions:* Our findings confirm the recovery of spermatogenesis but suggest a possible relationship between altered meiotic segregation and the abuse of androgenic anabolic steroids.

Keywords Anabolic steroids · FISH · Spermatozoa · TEM

Introduction

The effect of anabolic steroids on strength, body weight, oxygen uptake and spermatogenesis in mature males was described in 1972. Some years later, damage in spermatogenesis was recorded after administration of an anabolic steroid and recovery of semen characteristics was observed following the discontinuation of use [1].

The use of androgenic anabolic steroids (AASs) by athletes began in the 1950s and has increased since that time, but the benefit of these substances on performance and the damage to the health of athletes is still controversial. Changes in liver function, the development of liver tumors and the compromising of male reproductive function have been observed.

The non-medical use of AASs by male adolescents increased at a rate of 50% between 1991 and 1999. AASs induce hypogonadotrophic hypogonadism, associated with lower serum testosterone concentrations, testicular atrophy and impaired spermatogenesis.

Regarding this last issue, oligospermia, azoospermia, a decrease in testis size and in serum gonadotropins and testosterone levels were reported in a group of bodybuilders taking AASs compared to a control group [2]. However, it is known that damage in reproductive function, which may result in sterility, depends on the doses and duration of substance intake. Turek et al. [3] reported a case of reversibility of anabolic steroid-induced azoospermia. Concomitant abuse of AASs and Human Chorionic Gonadotrophin (HCG) impairs spermatogenesis in power athletes [4].

A study of sperm ultrastructure and meiotic segregation is still lacking in this field.

Transmission electron microscopy (TEM) is the only valuable tool for understanding the presence and the nature of the specific sperm malformations that cause human infertility.

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On the other hand, fluorescence *in situ* hybridization (FISH) is able to reveal the presence of meiotic errors in fertile and infertile men. The combination of these two techniques could better clarify the spermatogenic process.

We describe a case of a bodybuilder who used AASs for 6 years and resulted azoospermic at a first spermiogram. PCR analysis was performed in order to reveal the presence of Y microdeletions. After hormonal therapy, semen analysis was performed by light and TEM to observe ultrastructural sperm quality and by FISH to evaluate meiotic segregation.

Case report

A 34-year-old man was referred to our laboratory for semen analysis after 2 years of sexual intercourse without conception. His wife, aged 30 years, did not have any fertility problems. The patient told us that he had used massive doses of AASs, probably obtained from the black market, for six years to obtain a better physical appearance. Sexual development and physical examination were normal. Semen and urethral fluid were negative for microbial infection. Consanguinity was excluded. Lymphocyte karyotype was 46, XY. No history of infertility was reported. Serum hormone lev-

els (testosterone, cortisol, T3, T4, TSH, estradiol, FSH, LH, prolactin, and inhibin-B) were evaluated. Total testosterone, FSH, and LH levels were lower than standard range.

The patient provided written informed consent before inclusion in this study. 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. At the first semen analysis by light microscopy examination of ejaculate before and after centrifugation demonstrated the total absence of sperm. PCR screening of specific Y chromosomes according to EAA (European Academy of Andrology) guidelines did not show any deletions in the investigated regions.

After cessation of AASs abuse, the patient was treated with r-hFSH (Gonal-F, Serono, Italy) at dose of 150 IU sc on alternate days and hCG (Profasi, Serono, Italy) at dose of 100 IU twice a week for 6 months. At the end of treatment, semen analysis was performed and hormonal levels were reevaluated. Spermiogram evaluation showed a sperm count of $13 \times 10^6/\text{ml}$ and sperm progressive motility was 31%. The eosin Y test revealed 84% viable sperm and hormonal levels resulted within normal range. For the evaluation of sperm ultrastructure was performed TEM analysis. Three hundred

Fig. 1 TEM micrograph of sections of spermatozoa. The acrosomes are normal (nA) or misshapen (mA), the nuclei are normal (N) with condensed chromatin (cCh). The cross-sections of the tails showed normal axonemes (Ax) and accessory structures. $\times 17,000$

Fig. 2 TEM micrograph of a longitudinal section of sperm with normal nucleus (nN) and swollen acrosome (sA), mitochondria (M) are swollen and disassembled, the plasma membrane is broken (arrows). $\times 20,000$

sperm in ultra-thin sections were analyzed. Major submicroscopic characteristics were recorded by highly trained examiners who were blind to the experiment. TEM data was evaluated using the mathematical statistical formula of Baccetti et al. [5] which calculates the number of spermatozoa free of structural defects (“healthy”) and the percentages of three main phenotypic sperm pathologies: immaturity, necrosis, and apoptosis.

A high percentage of structurally normal spermatozoa (Fig. 1) were shown. The number of sperm probably de-

Table 2 Dual color FISH analysis for chromosomes 1, 9

Disomy frequency		Diploidy frequency
9, 9, 1	1, 1, 9	1, 1, 9, 9
0.183%	0.274%	0.320%

Note. In the patient, 2183 sperm were scored by dual colour FISH analysis.

void of ultrastructural defects was 1,207,603, almost ensuring natural fertility. In the sperm population with ultrastructural defects, the main alterations were related to necrosis: reacted or absent acrosomes, nucleus with disrupted chromatin, swollen and disassembled mitochondria and broken plasma membrane (Fig. 2).

Subsequently we carried out FISH sperm analysis according to Baccetti et al. [6] to evaluate aneuploidy frequency after the resumption of spermatogenesis (Table 1). We examined, by triple color FISH (chromosomes 18, X, Y), 4,131 sperm: only XY disomy was more frequent than in controls [3]; the frequency of other disomies and diploidies was normal. Dual color FISH for chromosomes 1 and 9 was also performed (Table 2). We examined 2,183 sperm, and the frequency of disomy in chromosomes 1 and 9 resulted higher (respectively 27% and 18%) compared to typical autosome values and the frequency of diploidy was comparable to that calculated using triple color FISH. Pregnancy was achieved at the end of therapy.

Discussion

In the present study, we report the case of an infertile patient, who had abused AASs. When semen analysis was performed for the first time, the patient resulted azoospermic. For this reason, a screening of Y chromosome microdeletion was carried out but no Y microdeletions were detected. Hormonal therapy was carried out and spermatogenesis was recovered after six months. TEM and FISH sperm analyses were carried out at the end of therapy.

Different studies have reported the effects of AASs on male reproductive function.

AASs induced hypogonadotropic hypogonadism, as suggested also by different authors, and the aging of the

Table 1 FISH analysis for chromosomes 18, X and Y

	Normal sperm X/Y-bearing	Disomy frequency				Diploidy frequency		
		18, 18	X, X	Y, Y	X, Y	18, 18, X, X	18, 18, Y, Y	18, 18, X, Y
Patient ^a	49.41%/49.60%	0.12%	0.07%	0.07%	0.242%	0.048%	0.096%	0.096%
Controls ^b		0.11% $\pm$ 0.003		0.23% $\pm$ 0.003			0.28% $\pm$ 0.004	

Note. The frequencies of nullisomies were: 18/0 = 0.096%; X/0 = 0.048%; Y/0 = 0.048%.

^aThe number of cells scored by triple FISH analysis was 4131.

^bSeven fertile men constituted the control group. Mean percentages ($\pm$ SD) of disomies and diploidies are reported for the chromosomes examined. The mean number of sperm scored by triple-FISH analysis was 5600 ± 260 .

hypothalamic-hypophyseal-gonadal axis, causing a decrease in GnRH secretion and consequently a decrease in the secretion of FSH and LH.

Torres-Calleja et al. [2] clarified the effect of AASs, evaluating changes in the hormonal profile and semen characteristics in a group of bodybuilders. Karila et al. [4] investigated the effects on male fertility of supra-physiological doses of AASs with or without the concomitant use of HCG under authentic conditions. They suggested that HCG maintains spermatogenesis in AAS abusers in a situation with no FSH stimulus, but that it produces significantly more abnormal and hypokinetic spermatozoa when used concomitantly with massive doses of AASs. In previous studies, morphological sperm quality was always evaluated by light microscopy, although subcellular sperm anomalies can only be detected by TEM. The mathematical formula developed by Baccetti et al. [5] calculates the percentage of sperm devoid of structural defects and the probability of sperm pathologies, highlighting fertility indices. In this case we observed a number of spermatozoa free of defects, almost assuring a normal fertility despite a reduced sperm count and progressive motility.

FISH analysis on sperm nuclei highlighted an increased frequency of sex chromosomes (XY) disomy, indicating a segregation anomaly at the first meiotic division and of chromosome 9 disomy.

In conclusion, we demonstrated a recovery of the spermatogenic process in this male who had used AASs, and ultrastructural semen analysis clearly demonstrated a sperm quality almost ensuring natural fertility, which was achieved

after therapy with gonadotropins. On the other hand, our study also shows, for the first time, anomalies in the meiotic process in this type of case. Therefore, the observed genetic damage involving sperm chromosome segregation could be a sign of alarm for subjects that habitually use steroids.

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