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Chromosomal Aneuploidy in Azoospermic Men

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ABSTRACT The aim of this study was to estimate the prevalence of chromosome abnormalities in patients with azoospermia, in our material. Preoperative evaluation included routine andrological investigations with 2 semen analysis, ultrasound, hormonal and genetic examinations. In the last three years, 73 biopsies were performed for testicular sperm extraction in 71 patients. Non-obstructive azoospermia was diagnosed in 53 patients (79%). Karyotyping was performed on lymphocyte preparations of 36 men with diagnosis of infertility. In order to obtain exact diagnosis, cytogenetic methods (QFQ-, GTG-, CBG-band and FISH analysis) were complemented with molecular genetic techniques. Patients were included in the assisted reproduction programme on the base of their genetic results. The most characteristic cases were sex numerical deviations, such as XXY (3 cases), XYY (2 cases) and mosaic X0 syndromes (1 case).

INTRODUCTION

Data from the Hungarian Central Statistical Office (KSH) show that infertility affects about 150 000 couples in Hungary, which means that one in seven couples have problems in conceiving. The women were thought the main cause of the childless partnerships in the past, but the recent examinations showed that in infertile couples, the female and the male factor can be identified equally (40-40%) and both in 20% (Foresta et al. 2001; Huynh et al. 2002; Zhang et al. 2004).

A marked decline in male reproductive health, and an increase in the population of subfertile males have been demonstrated worldwide. Both genetic and environmental factors are considered to be responsible for this decline (Dada et al. 2004). According to WHO, the number of the spermatozoon in the normal spermiogram were 80 millions / ml in 1960 and currently 20 millions / ml. The cause of male infertility is unknown in more than 50% of cases. A few research studies have focused on the possible genetic aetiologies (Dada et al. 2004; Foresta et al. 2001). The improvement of assisted reproductive techniques has contributed to the development of research in this field.

Chromosomal abnormalities (10-15%) are one of the major causes of male infertility (Patsalis et al. 2002; Pienna Videau et al. 2001). Numerical or structural chromosomal anomalies can be found in the background in 5% of the cases. Approximately 80% of these cases are due to sex chromosome abnormalities, in about 2% it occurs mixed with autosomal abnormalities (Huynh et al. 2002; Siffroi et al. 2000; Visootsak et al. 2001). Between 10% and 20% infertile men carry microdeletions in the euchromatic region of the long arm of the Chromosome Y. These microdeletions define three regions of Yq11 such as Azoospermia factors –AZFa, AZFb, and AZFc (Patsalis et al. 2002, Siffroi et al. 2000; Silber et al. 2002).

The aim of our study was to investigate chromosomal abnormalities in infertile men prior to medicine treatment or surgery helping the couples waiting for the assisted reproduction.

MATERIALS AND METHODS

Our survey included 71 men with diagnosis of infertility during January 2001 – August 2005 (no spontaneous pregnancy developed following unprotected intercourses over more than one year period of time). Preoperative evaluation included routine andrological investigation with physical examination, 2 semen analyses, ultrasound, assessment of hormone levels (FSH, LH, prolactin, testosterone) and

genetic examination. Indication for testicular sperm retrieval included azoospermia or very severe oligozoospermia in vast majority of patients.

73 biopsies were performed in 71 patients for testicular sperm extraction (TESE). The men were 24-53 years of age with a median of 34 years.

In men with azoospermia or very severe oligozoospermia - the most severe forms of male factor infertility - various techniques are used for surgical sperm retrieval. The routine procedure for sperm retrieval is the bilateral open multiple mapping biopsies. Same techniques are used for all patients with obstructive and non-obstructive azoospermia. Abbreviation used for assisted reproductive method is: CRYO-TESE-ICSI (cryopreservation- sperm extraction-intracytoplasmic injection).

In the last three years, traditional cytogenetic methods and molecular genetic techniques were used for chromosomal analysis of 36 patients. Cytogenetic and FISH studies were performed on lymphocytes of peripheral blood. Initiation of mitosis of the lymphocytes was performed in vitro with bacto phytohemagglutinin-P and M. After 72-hour growth chromosomes were stopped in mitotic metaphase by colchicine.

The used chromosomal staining methods were the following: GTG, QFQ, CBG banding techniques and fluorescence *in situ* hybridization (FISH) analysis with Y painting probes labelled with spectrum green and X painting probe with spectrum orange labelling. Deletions of the Y chromosome were diagnosed by polymerase chain reaction (PCR) using Y specific sequence tagged sites (STS) spreading the AZFa, AZFb and AZFc loci.

RESULTS

Non-obstructive azoospermia was diagnosed in 53 patients (79%) and obstructive 13 patients (21%). Testicular cancer was found incidentally in one patient. Chromosomal aberrations were found in 6 patients (8%). Table 1 show the andrological, histological and cytogenetical findings of the studied 6 patients:

Klinefelter-syndrome were found in three patients, and two patients (P1,P2) had non-mosaic 47XXY karyotype (Fig. 1). In case of patient 3, the genetic study showed 47XXY karyotype in most of the counted metaphases mitotic chromosome groups. In some cells we found 49XXXXY karyotype (Fig. 2). All patients were tall, and one man (P3) had gynecomastia. The testes were small and firm, and an elevated FSH level was found in these men. In three patients, spermatozoa were not detected in the testicular biopsy specimens.

In two patients (P4,P5), the genetic study showed an extra Y chromosome in all metaphases* (Fig. 3). One of them had spermatozoa sufficient for ICSI in tissue samples from both testes, and also in the ejaculate. A successful ICSI treatment was performed and followed by a normal pregnancy and delivery. The couple has a healthy 2-year-old daughter. In the other patient with atrophy of the testicals, histology revealed only a few mature spermatozoa.

One patient (P6) showed a mixed karyotyping. In more than 90% of the cells the Y chromosome was missing (45X) (Fig. 4), while the rest of the cells carried a deleted Y chromosome that consisted of the Y short arm and the centromere, but most of Yq was deleted. A

Table 1: Andrological, histological and cytogenetical findings in 6 patients

Patients	*Age*	*Height*	*Weight*	*Testis*	*Ejaculate*	*FSHI U/L*	*LHI U/L*	*Testosteroneng/ml*	*Histology Of testicular biopsy*	*Cytogenetics*
1	31	185	70	smaller, firm	0 M/ml	elevated			spermatozoa were not found	47XXY
2	29	178	84	smaller and firm	0 M/ml	32,72	13,95	2,05	spermatozoa were not found	47XXY
3	32	189	110	low volume, firm	0 M/ml	16,51	8,75	3,93	Sertoli cell only syndrome	47XXY/ 49XXXXY
4	28	190	80	left: normal, right: smaller, atrophied	0-1 M/ml	16,51	6,57	3,53	adequate spermatozoa	47XYY
5	42	185	92	limp, left: varicocele	0 M/ml	12,03	9,11	7,5	only a few spermatozoa	47XYY
6	36	160	58	normal	0 M/ml	27,38	7,04	5,32	maturation arrest, Sertoli cell only syndrome	45X/46XderY

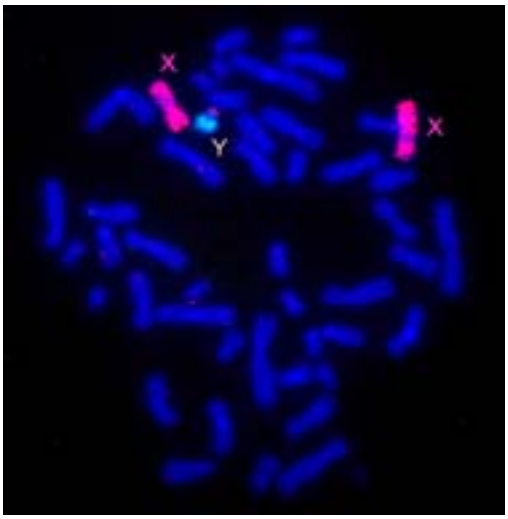

Fig. 1. P1 patient with Klinefelter-syndrome (FISH analysis: der Y is labelled with green and X chromosome is red)

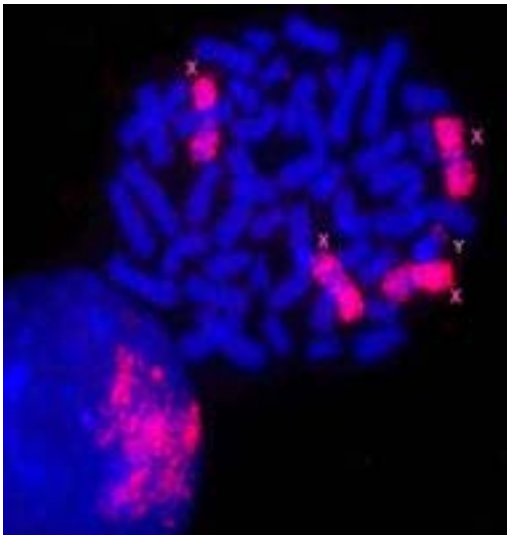

Fig. 2. 49XXXXY cell from P3 patients with FISH

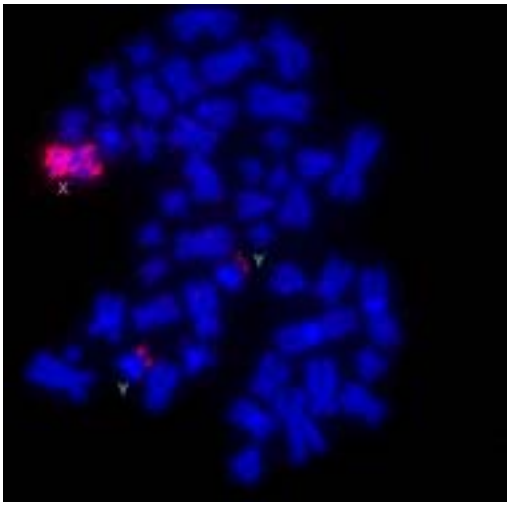

Fig. 3. Extra Y chromosome from P4 patient with FISH analysis (the red X painting probe also dyed the Yq)

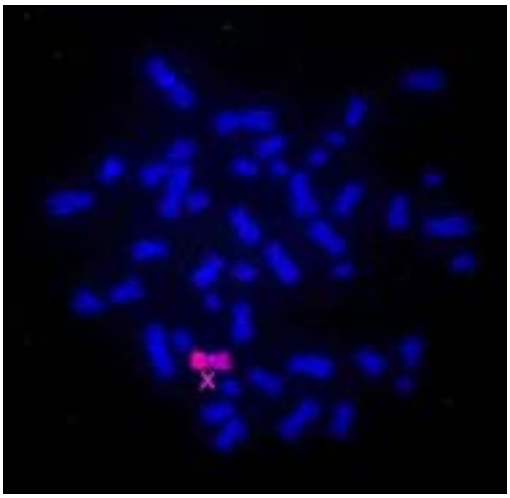

Fig. 4. Mosaic 45X0/46XY patient 6: more than 90% of the chromosome group contain only one X chromosome (labelled with red)

representative example of such Y derived material is shown in Figure 5. The PCR analyses confirmed the deletion of the AZFb and AZFc regions of the Y chromosome (Table 2). Spermatozoas sufficient for ICSI were not found.

DISCUSSION

Assisted reproductive technologies (ART), including intracytoplasmic sperm injection (ICSI) have made conception possible for many couples with severe male factor infertility (Komori et al. 2002). In some patients, testicular sperm extraction is a plausible option to overcome infertility. However, in spite of modern reproductive technologies, it is hopeless in patients with maturation arrest or "Sertoli cell only" syndrome. In our study we found sufficient spermatozoa

Table 2: Results of the sequence tagged site (STS) PCR analysis of patient 6 (present+, absent-)

sY84	sY86	sY114	sY127	sY134	sY143	sY152	sY157	sY158	sY254	sY255
AZFa			AZFb					AZFc		
+	+	-	-	-	-	-	-	-	-	-

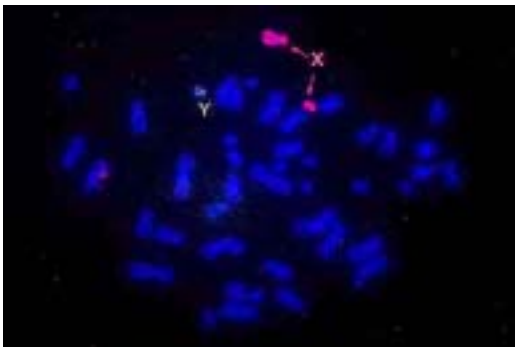

Fig. 5. Mosaic 45X0/46XY patient 6: less than 10% of the metaphase contain an X (red) and a derivated Y chromosome (green). The X chromosome was broken in this cell.

for ICSI in 36 cases (54%). Among those 6 patients who had chromosomal abnormalities only one had sufficient spermatozoa.

The XXY syndrome is the most common genetic cause of human male infertility. The prevalence of the syndrome is 0,6-1‰ in the general population and about 10% in the infertile male population (Bielanska et al. 2000; Huynh et al. 2002; Lanfranco et al. 2004). Approximately 80% of the cases are due to the congenital numerical chromosome aberration (47 XXY), and 20% of the cases display higher-grade chromosome aneuploidies, mosaicism or structurally abnormal X chromosomes. In our study, the Klinefelter-syndrome was also the most frequent chromosome aberration similar to literature data. We found non-mosaic 47XXY syndrome in two patients and 47XXY/49XXXXY mosaicism in one patient. Each patient was azoospermic. There are few reports of very low rate spermatogenesis in occasional patients with Klinefelter syndrome, and very rare cases from paternity tests. Earlier we have reported one case in a legal paternity action. (Bielanska et al. 2000; Bujdosó et al. 1976; Huynh et al. 2002; Lanfranco et al. 2004; Pienna Videau et al. 2001; Visootsak et al. 2001).

The XYY syndrome is also a common abnormality; with a frequency of 1/1,000 in live births. The majority of 47XYY males are fertile and have chromosomally normal offsprings. However, an increased risk for offspring with chromosomal abnormalities has been suggested for these men, because XYY males have an increased frequency of sex chromosome disomic spermatozoa **(**Lim et al. 1999**;** Martin et al. 2001**;** Wang et al. 2000**;** Zhang et al. 2004). We found XYY syndrome in two patients, one of them had spermatozoa sufficient for ICSI both in the semen and testis. In the other patient with atrophy of the testicals, histology revealed only a few mature spermatozoa.

Aberrations on the Y chromosome are currently found in about 10% of infertile and in 2% of fertile men. The vast majority of deletions associated with complete absence of germ cells (azoospermia) or with severe oligozoospermia are cytogenetically undetectable. Due to infertile phenotype, most deletions are de novo. Abnormality of the Y chromosome seems to be frequently associated with 45X / 46XY mosaicism (Patsalis et al. 2002; Siffroi et al. 2000; Simoni et al. 2004; Telvi et al. 1999). A wide phenotypic spectrum has been reported presenting 45X/46XY mosaicism, ranging from females with Turner stigmata and male or female pseudo-hermaphroditism to almost normal male development (Álvarez-Nava et al. 2003; Huang et al. 2002; Telvi et al. 1999). The extent of male or female differentiation mainly depends on the prevalence of the 45X cells.

In our study, Y chromosome deletion was found in one case. This patient had a very high percentage of 45X0 cells (over 90%). The 46XY cells (less than 10%) included a small, deleted Y chromosome. The andrological and histological examination revealed sperm neither in the semen nor in the testis. He had a normal-appearing male phenotype with short stature. The cause of growth failure is unclear in the 45X/46XY patients (such as in Ullrich-Turner syndrome), but probably no positive relationship exists between the percentage of mosaic cells and the short stature. The aetiology of short stature may be explained by chromosome imbalance or specific genetic factors such as short stature homeobox (SHOX) gene (Richter-Unruh et al. 2004).

Conventional cytogenetic techniques as the karyotyping is needed to evaluate which men are candidates for genetic counselling and

assisted reproductive technologies. With the precise diagnosis of a disorder we could save these patients from a long, expensive examinations, and unsuccessful procedures. Therefore, the number of unsuccessful ICSI cycles may be reduced. Moreover, in case of a male with sex chromosome anomaly, we have to consider a higher risk of fathering a child with autosomal or gonosomal aneuploidies after a successful fertilisation treatment (Bielanska 2000; Lanfranco et al. 2004; Lim et al. 1999; Patsalis et al. 2002; Wang et al. 2000; Zhang et al. 2004). In case of confirmed microdeletion, kryopreservation of the sperm in young age should be offered since the number of the sperm dramatically decreases with the age. The infertile men with non-mosaic microdeletion of the Y chromosome will inevitably transmit the mutation to the male offspring (Komori et al. 2002; Patsalis et al. 2002; Silber et al. 2002).

The genetic diagnosis may help to prevent the transmission of male infertility to the next generations, and the prospective parents should be advised about the risk of chromosomal aberration in the next generations through genetic counselling.

If no cytogenetic or Y chromosomal abnormality is found in an infertile male patient, this does not obviate the likelihood of a genetic cause for his azoospermia or severe oligozoospermia. With the available examinations and modern techniques we could find the most adequate treatment for every patient, and we can rely on that these patients will have healthy offsprings through natural way or assisted reproduction.

*Patient 5 was received from the Hungarian Military Hospital, Department of Urology, Budapest

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