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·Review·

FISH studies of chromosome abnormalities in germ cells and its relevance in reproductive counseling

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Abstract

Chromosome abnormalities are one of the major causes of human infertility. In infertile males, abnormal karyotypes are more frequent than in the general population. Furthermore, meiotic disorders affecting the germ cell-line have been observed in men with normal somatic karyotypes consulting for infertility. In both cases, the production of unbalanced spermatozoa has been demonstrated. Basically addressed to establish reproductive risks, fluorescence *in situ* hybridization (FISH) on decondensed sperm heads has become the most frequently used method to evaluate the chromosomal constitution of spermatozoa in carriers of numerical sex chromosome abnormalities, carriers of structural chromosome reorganizations and infertile males with normal karyotype. The aim of this review is to present updated figures of the information obtained through sperm FISH studies with an emphasis on its clinical significance. Furthermore, the incorporation of novel FISH-based techniques (Multiplex-FISH; Multi-FISH) in male infertility studies is also discussed. *(Asian J Androl 2005 Sep; 7: 227–236)*

Keywords: chromosome abnormalities; FISH; germ cells; male infertility; reproductive counseling

1 Introduction

Genetic abnormalities have long been accepted as an important cause of human infertility and are suspected to be present in 30 % of the patients consulting for fertility problems [1]. As a consequence, genetic studies, either cytogenetic or addressed to the detection of specific gene mutations, have been incorporated in most male infertility screening protocols, and are of great importance for the affected couples seeking reproductive counseling.

Focusing on cytogenetic studies, a study of the karyotype is usually included in the basic clinical evaluation. The high incidence of constitutional chromosomal abnormalities in infertile patients in relation to the general population [2–4] justifies its application and allows the diagnosis of approximately 7 % of the cases of male infertility [3]. On the other hand, meiotic cytogenetic studies directed towards the detection of abnormalities, which exclusively affect the germinal line, not detectable through the study of the somatic karyotype and performed through the analysis of testicular tissue, are less commonly requested. However, it has been shown that approximately 6 % of the patients with a normal somatic karyotype who seek advice for infertility present meiotic alterations in their spermatogenic cells [5]. During the last

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years, probably because of the use of testicular material for assisted reproductive techniques, meiotic studies have recovered their deserved space in the diagnosis of human male infertility. Substantial information has been derived from more recent reports, showing meiotic abnormalities in 17.5 % of males with severe oligoasthenoz oospermia [6] and that this figure can increase up to 27 % in normozoospermic patients with one or more previous IVF failures [7].

As mentioned, chromosomal abnormalities (either constitutional or limited to the germ cell line) interfere with gametogenesis and may result in the production of gametes with chromosomal abnormalities that, in turn, increase the risk of affected offspring.

With the introduction of fluorescent *in situ* hybridization (FISH) techniques, the possibilities of analysis of male germ cells are notably increased. In this context, FISH studies in spermatozoa were promptly incorporated into the study of infertile patients [8–12]. The application of combinations of DNA probes appropriate for each specific study, preceded by a protocol of spermatic chromatin decondensation, allows to obtain reliable data on the frequency of chromosomal abnormalities in the spermatozoa and valuable information for guiding reproductive counseling in each case.

Furthermore, with the objective of obtaining more detailed cytogenetic information of the whole spermatogenic process (spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa), the combination of different FISH-based techniques on testicular tissue can also be used [13]. Analyses by means of Multiplex-FISH (M-FISH), using DNA probes directly labeled with a combination of five different fluorochromes, thus allowing the obtention of 24 different color patterns and making possible the identification of all chromosomes in metaphase I and metaphase II cells, are especially promising [14].

This paper reviews the results obtained from the application of FISH methodologies in the cytogenetic characterization of spermatogenesis in three groups of individuals: 1) carriers of numerical abnormalities for the sex chromosomes; 2) carriers of structural chromosomal abnormalities; and 3) infertile individuals with a normal karyotype.

The behavior of the chromosomes involved in the abnormalities throughout the meiotic process, the risk of transmission to the offspring and the possible implications in reproductive counseling in each group will be detailed.

2 Carriers of numerical sex chromosome abnormalities

Carriers of sex chromosome abnormalities are frequently seen in andrology services and fertility clinics. Sperm FISH studies carried out in these patients show an increase in the incidence of sex chromosome disomies (Table 1).

In apparently non-mosaic Klinefelter individuals, the average incidence of disomies for sex chromosomes is 6.29 % (range 1.36 %–25 %), while in 46,XY/47,XXY mosaics it is 2.54 % (range 0 %–7 %) (Table 1). Sperm FISH studies in 47,XYY individuals show that 3.74 % (range 0.11 %–14.36 %) of the spermatozoa analyzed (Table 2) are carriers of an extra sex chromosome.

The mechanisms by which these increases are produced are not well known yet. Although the early studies suggested the possible entry of the XXY aneuploid line in meiosis [36, 37], more recent studies indicate that these cells cannot enter the meiotic process [26, 38]. In these individuals, the observed increase of disomies could be justified by an abnormal testicular micro-environment, probably related to an increase of FSH, which would affect chromosomal segregation in a euploid 46,XY cell line. In 47,XYY individuals, although some studies suggested that the extra Y might be lost in the early stages of spermatogenesis [39–43], other studies [26, 30, 44] have shown it clear the capacity of some aneuploid cells to initiate and complete meiosis, producing aneuploid gametes.

Due to the relatively low frequency of disomic spermatozoa observed and the clinical features of numerical sex chromosome syndromes, some authors debated on performing routine sperm FISH analysis in these individuals [45, 46]. In our opinion, the variability shown by the different studies (Tables 1 and 2) indicates the need for the individualized analysis for each particular case. For example, according to the results of the studies shown in Table 1, it is evident that in a 47,XXY patient with an incidence of 21.71 % of disomies for the sex chromosomes [18], reproductive counseling will be quite different from that of another individual, also characterized as non-mosaic Klinefelter, but with a much lower disomy incidence (1.36 %) [22].

3 Carriers of structural chromosomal abnormalities

Table 1. Percentage of chromosomal abnormalities in spermatozoa of Klinefelter's syndrome patients. [a]No statistical analysis has been performed; [b]statistically significant *vs* internal controls.

Reference	Authors	Karyotype	XY	XX	YY	Diploid
15	Chevret *et al.* (1996)	46,XY/47,XXY	2.09[b]	0.11	0.003	0.33
16	Martini *et al.* (1996)[a]	46,XY/47,XXY	1.30	0.5	0.7	–
17	Guttenbach *et al.* (1997)	47,XXY	1.36[b]	1.22[b]	0.09	0.23[b]
18	Foresta *et al.* (1998)	47,XXY	14.58[b]	6.92	0.21	0.05
		47,XXY	10.03[b]	3.34	0.09	0.03
19	Kruse *et al.* (1998)[a]	46,XY/47,XXY/48XXXY	5	2	–	–
20	Estop *et al.* (1998)[a]	47,XXY	25[b]	–	–	4.2[b]
21	Lim *et al.* (1999)	46,XY/47,XXY	0.41[b]	0.29[b]	0.06	1.70[b]
22	Rives *et al.* (2000)	47,XXY	0.54[b]	0.45[b]	0.37[b]	0.23[b]
		46,XY/47,XXY	0.62[b]	0.24[b]	0.20	0.36[b]
23	Morel *et al.* (2000)	46,XY/47,XXY	1.3[b]	0.71[b]	–	0.24[b]
		46,XY/47,XXY	1.73[b]	0.86[b]	0.86[b]	0.25[b]
24	Levron *et al.* (2000)[a]	47,XXY (5)	0.89	1.79	0.89	–
25	Bielanska *et al.* (2000)[a]	46,XY/47,XXY	2.23	1.12	0.56	0.84
26	Blanco *et al.* (2001)	47,XXY	1.37[b]	–	–	1.37[b]
		46,XY/47,XXY	–	–	–	–

Table 2. Percentage of chromosomal abnormalities in spermatozoa of 47,XYY patients. [a]No statistical analysis has been performed; [b]statistically significant *vs* internal controls.

Reference	Authors	XY	XX	YY	Diploid
27	Han *et al.* (1994)	0.25	0.30	0.40	3.35[b]
28	Mercier *et al.* (1996)	9.37[b]	0.34	4.65[b]	0.11
16	Martini *et al.* (1996)[a]	2.30	2	0.80	–
		5.40	2.70	2.30	–
29	Chevret *et al.* (1997)	0.24	0.02	0.08[b]	0.23[b]
		0.52	–	0.19[b]	0.13[b]
30	Blanco *et al.* (1997)	0.30[b]	0.15	1.01[b]	0.30
31	Mennicke *et al.* (1997)[a]	0.80	0.70	0.50	–
		1	1.2	1	–
		3.11	0.31	1.02	0.08
32	Martin *et al.* (1999)	0.55[b]	0.08	0.03	0.12
33	Morel *et al.* (1999)[a]	3.01	1	1.64	0.15
34	Shi and Martin (2000)	0.44[b]	0.05	0.07[b]	0.33
26	Blanco *et al.* (2001)	0.11	–	–	0.44
35	Rives *et al.* (2003)	3.41[b]	0.54[b]	1.23[b]	1.49[b]
		0.83[b]	2.20[b]	1.65[b]	1.38[b]

The development of locus specific and subtelomeric DNA probes has allowed the analysis of chromosome segregation in carriers of inversions (pericentric and paracentric), Robertsonian translocations and reciprocal translocations. These studies confirmed that carriers of structural chromosomal reorganizations produce, to a greater or a lesser extent, chromosomally unbalanced spermatozoa depending on the characteristics of the reorganization (Tables 3, 4 and 5). If these gametes fertilize an oocyte, the resulting embryos, depending on the chromosome regions implicated, can give rise to abortions or offspring affected by chromosomal abnormalities.

In patients, carriers of pericentric inversions, the frequencies of abnormal spermatozoa vary in function of the size of the inverted region, ranging between 0.67 % and 54.30 % (Table 3). For carriers of Robertsonian translocations, unbalanced spermatozoa range is between 7 % and 36 % (Table 4), and the range is between 29.37 % and 70.20 % in carriers of reciprocal translocations (Table 5). Among the different factors which condition chromosome segregation, the chromosomes involved in the reorganization and the characteristics of the reorganized regions (such as the location of the breakpoints involved in the rearrangement) seem to be especially important.

Furthermore, the presence of structural reorganizations may produce an interchromosomal effect (ICE), characterized by the abnormal behavior of one or more bivalents not involved in the reorganization, which could give rise to abnormal spermatozoa for these chromosomes. In this sense, diverse FISH studies where this phenom-

Table 3. Percentage of chromosomally abnormal spermatozoa in carriers of pericentric inversions.

Reference	Authors	Inversion	unbalanced (%)
47	Jaarola *et al.* (1998)	inv(1)(p31q12)	0.67
		inv(8)(p23q22)	13.7
48	Anton *et al.* (2002)	inv(6)(p23q25)	54.3
49	Yakut *et al.* (2003)	inv(1)(p36q32)	17.5
50	Mikhaail-Philips *et al.* (2004)	inv(2)(p23q33)	44.1

Table 4. Results of segregation and interchromosomal effect (ICE) in carriers of Robertsonian translocations. N/B: normal/balanced; A/U: abnormal/unbalanced; Alt = alternate; Adj = adjacent; ne = not evaluated.

Reference	Authors	Translocation	Type of segregation		ICE
			N/B (Alt)	A/U(Adj)	
51	Rousseaux *et al.* (1995)	t(14q;21q)	72.20	18.01	+
31	Mennicke *et al.* (1997)	t(21q;22q)	60	36	ne
52	Honda *et al.* (2000)	t(14q;21q)	88.42	11.25	ne
53	Escudero *et al.* (2000)	t(13q;14q)	73.60	23.30	ne
		t(13q;14q)	77.40	19.10	ne
54	Blanco *et al.* (2000)	t(13q;22q)	ne	ne	–
55	Morel *et al.* (2001)	t(13q;14q)	81.34	18.06	+
		t(13q;14q)	82.60	16.32	+
		t(13q;14q)	88.90	10.08	–
56	Frydman *et al.* (2001)	t(13q;14q)	91	9	ne
		t(13q;14q)	90	10	ne
		t(13q;14q)	87.10	12.90	ne
		t(14q;21q)	91.30	8.70	ne
		t(14q;21q)	92.80	7.20	ne
		t(14q;21q)	93	7	ne
57	Acar *et al.* (2002)	t(21q;21q)	ne	ne	–
58	Anton *et al.* (2004)	t(13q;14q)	86.48	12.56	–
		t(13q;14q)	87.49	12.17	–
		t(13q;14q)	83	14.53	+
		t(13q;14q)	84.53	14.17	–
		t(13q;14q)	88.13	11.40	–
		t(13q;14q)	88.23	11.11	+
		t(13q;14q)	87.73	11.63	–

enon has been evaluated show a positive ICE in 38.46 % of the studied Robertsonian translocations (Table 4), and approximately 34.5 % in reciprocal translocations (Table 5).

Sperm FISH studies in structural chromosome reorganization carriers allow to infer the meiotic behavior of the rearranged chromosomes and the final outcome in spermatozoa. However, the importance of improving our knowledge of the meiotic process in these individuals has led to the application of M-FISH techniques for an in-depth cytogenetic analysis, thus allowing the evaluation of all bivalents or multivalents in metaphase I, making it possible to analyze the meiotic configuration of the chromosomes involved in a given reorganization and to evaluate the occurrence of interchromosomal effects [14, 77].

Table 5. Results of segregation and interchromosomal effect (ICE) in carriers of reciprocal translocations. N/B = normal/balanced; A/U = abnormal/unbalanced; Alt = alternate; Adj = adjacent; ne = not evaluated.

Reference	Authors	Translocation	Type of segregation		ICE
			N/B (Alt)	A/U(Adj I + Adj II + 3:1)	
59	Lu *et al.* (1994)	t(2;4;8)(q23;q27;p21)	ne	ne	–
60	Rousseaux *et al.* (1995)	t(6;11)(q14;p14)	ne	ne	+
		t(6;11)(q14;p14)	ne	ne	+
		t(2;14)(p23.1;q31)	ne	ne	+
61	Van Hummelen *et al.* (1997)	t(1;10)(p22.1;q22.3)	48.09	51	–
62	Blanco *et al.* (1998)	t(5;8)(q33;q13)	45.12	51.84	–
63	Estop *et al.* (1998)	t(2;18)p21;q11.2)	43.60	53.10	ne
		t(8;9)(q24.2;q32)	44.40	53.50	ne
64	Martini *et al.* (1998)	t(3;11)(q27.3;q24.3)	44.30	51.40	–
65	Estop *et al.* (1999)	t(11;22)(q23;q11)	27.40	70.20	ne
66	Cifuentes *et al.* (1999)	t(5;7)(q21;q32)	49.70	50.30	–
67	Honda *et al.* (1999)	t(3;9)(q26.2;q32)	52.49	47.24	–
		t(3;9)(p25;q32)	47.25	52.48	–
68	Giltay *et al.* (1999)	t(Y;16)(q11.21;q24)	51	48	–
54	Blanco *et al.* (2000)	t(3;15)(p25;q15)	ne	ne	+
		t(Y;7)(q13;p11)	ne	ne	–
69	Estop *et al.* (2000)	t(10;12)(p26.1;p13.3)	ne	ne	–
		t(2;18)(p21;q11.2)	ne	ne	–
		t(3;19)(p25;q12)	ne	ne	–
		t(11;22)(q23;q11)	ne	ne	–
		t(3;4)(p25;p16)	ne	ne	–
		t(8;9)(q24.2;q32)	ne	ne	–
		t(10;18)(q24.1;p11.2)	ne	ne	–
		t(4;10)(q33;p12.2)	ne	ne	–
70	Morel *et al.* (2001)	t(X;Y)(p22.3;q11)	ne	ne	+
71	Oliver-Bonet *et al.* (2001)	t(4;8)(q28;p23)	30.5	68.50	+
72	Geneix *et al.* (2002)	t(17;22)(q11;q12)	19	65.50	ne
73	Oliver-Bonet *et al.* (2002)	t(1;13)(q41;q22)	41.6	58.40	–
		t(3;19)(p21;p13.3)	39.1	60.90	+
74	Rives *et al.* (2003)	t(9;10)(q11;p11.1)	56.25	43.74	–
75	Baccetti *et al.* (2003)	t(10;15)(q26;q12)	32.8	65.80	+
76	Morel *et al.* (2004)	t(7;8)(q11.21;cen)	56.7	43.26	+
		t(7;8)(q11.21;cen)	62.84	36.88	+

In summary, the combination of meiotic studies and cytogenetic sperm analysis can help to better establish appropriate reproductive advice for these patients. The variability observed in the studies reported from individual carriers of inversions (Table 3) and from carriers of Robertsonian and reciprocal translocations (Tables 4 and 5) recommends that an exhaustive study could be carried out in infertile men to facilitate the subsequent application of the most appropriate reproductive strategy.

4 Infertile individuals with a normal karyotype

The majority of infertile individuals have a normal somatic karyotype, but show altered semenograms frequently. Furthermore, it has been reported that the frequencies of aneuploid and diploid sperm present in a

given semen sample are directly related to the reduction in the number and progressive motility of sperm [6,10], high levels of FSH [6] and previous IVF failures in the case of normozoospermic patients [7].

In normal individuals disomies are around 0.1 % for most autosomes and 0.3 % for chromosome 21 and the sex chromosomes. Increases in sperm aneuploidies in individuals showing oligoasthenoteratozoospermia (OTA) have been reported by many authors [8,10,78–91]. Furthermore, an increase in aneuploid sperm has been detected in individuals with alterations of any of the basical semen parameters: sperm number [89, 92], sperm motility [10] and sperm morphology [80, 89, 93–97]. Meiotic errors, either affecting synapsis during prophase I or meiotic recombination [98–100], can be the starting point for the production of these chromosomally unbalanced spermatozoa. These meiotic abnormalities have been related to mutations of meiosis-specific-genes involved in synaptic events, DNA recombination and DNA repair [101], as well as environmental factors [5, 38].

It is well known that, in assisted reproduction, when no sperm cells are present in the ejaculate, those coming from the epididymis or from the testicle are used. In the few cases in which sperm FISH studies have been undertaken, no significant differences in the frequencies of aneuploidy and diploidy from those observed in ejaculate sperm have been reported for epididymal sperm. Nevertheless, these results differ in testicular spermatozoa where the reported incidence of diploidy and disomies (especially for the sex chromosomes) is higher [102–104].

However, compilation of sperm FISH data in this group of patients (infertile with normal karyotype) reveals a great heterogeneity, and important interindividual variations in the results obtained. Thus, it is in these individuals that the application of complementary studies combining different FISH techniques (Multi-FISH in interphase nuclei and Multiplex-FISH in metaphase I and metaphase II spermatocytes) can provide more useful information about the entire spermatogenic process (from spermatogonia to spermatozoa). Furthermore, the possibility of identifying and analyzing all the chromosomes in metaphases I and metaphase II will provide an accurate evaluation of the chromosomes affected by synaptic abnormalities and of their meiotic behavior [14]. From a clinical point of view, in the group of patients-frequent candidates in assisted reproduction programs-the application of the different techniques available would allow better reproductive counseling.

5 Conclusion

As shown in this review, spermatozoa of infertile individuals showed a greater incidence of chromosomal abnormalities than those of the fertile population. FISH studies in decondensed sperm nuclei became a notable advance in the study and diagnosis of male infertility and were widely incorporated both in the clinical practice and in the field of basic research.

The possibility of combining different FISH-based techniques opens new prospects for a better understanding of the entire spermatogenic process. Furthermore, these approaches offer novel possibilities for exhaustive cytogenetic analyses of the meiotic process, that could allow a better understanding of the meiotic process and of the consequences of meiotic abnormalities.

From a clinical point of view, the result of pooling all the data obtained from these analyses could help in the reproductive counseling offered to these patients. For instance, many groups of infertile patients are candidates for preimplantation genetic diagnosis [105,106], reorienting the chromosomal screening of the embryos.

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