Running title: Sperm FISH studies

Title: Role of sperm FISH studies in infertile patients: indications, study approach and clinical relevance

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22 **Capsule**

23 This article reports the indications, the study approach and the clinical relevance of
24 sperm FISH analysis of infertile men.

25

Abstract

Objective: To determine the group of infertile patients that would benefit from sperm FISH analysis, the number of chromosomes to be analyzed and the diagnostic interpretation of the results.

Design: Retrospective study.

Setting: Universitat Autònoma de Barcelona.

Patient(s): 319 infertile men.

Intervention(s): Semen samples were processed for FISH.

Main Outcome Measure(s): The frequencies of chromosomal abnormalities for chromosomes 13, 18, 21, X and Y were compared to the seminogram, the somatic and meiotic karyotype, and the age.

Result(s): The highest percentages of patients with an increased rate of sperm chromosome abnormalities were found in the oligozoospermic (50%), oligoasthenozoospermic (33.3%) and oligoasthenoteratozoospermic individuals (21%). Low sperm count correlated with the percentage of chromosome abnormalities. The 14% of the individuals with a normal somatic karyotype had an increased rate of sperm chromosome abnormalities. This percentage was higher in altered somatic karyotype patients (36%) and in those with meiotic abnormalities (26%).

Conclusions: Sperm FISH is indicated when the oligo condition is present and in individuals with an abnormal somatic or meiotic karyotype. The analysis of chromosomes 21, X and Y is enough to identify at-risk individuals. Any significant increase in chromosomal abnormality rates should be taken into consideration and patients should be considered at-risk.

Key words: chromosome abnormalities, clinical relevance, indications, male infertility, sperm FISH, study approach.

Introduction

Aneuploidies are the most frequent chromosomal abnormalities in humans, and are detected in 35% of miscarriages, 4% of stillbirths and 0.3% of live births (1). The majority are the result of meiotic errors during the process of gametogenesis in the parents. In men the study of the origin of these errors has revealed abnormal meiotic recombination (2) which, in addition to being related to the production of aneuploid gametes, could give rise to different degrees of meiotic arrest (3). Although meiotic studies are occasionally carried out in the context of clinical diagnosis (2) one of the protocols which has been most widely and most rapidly incorporated is fluorescent in situ hybridization (FISH) in spermatozoa, a technique that provides an estimate of the frequencies of chromosomal abnormalities.

Several authors have reported a high number of chromosomal abnormalities in spermatozoa from patients with an altered seminogram (4-8). This result has been related to the diminished fertility of the patients and to an increased risk for the transmission of abnormalities. However, there is a great variability among studies, either due to the number of individuals studied, the criteria for classifying them, the characteristics of the control group used, the criteria used to assess samples, or even the statistical comparisons performed (9). Thus, from published data, it is difficult to identify which group of infertile patients would most benefit from a sperm FISH study.

Another controversial aspect of sperm-FISH studies concerns the number and the chromosomes to be evaluated to ensure an appropriate reproductive counseling. To date, the most widely-studied chromosomes have been X, Y, 13, 18 and 21. However, a considerable number of centers have started to include other chromosomes in their studies, the idea being that the more information is available the more accurate the diagnosis will be. This has raised doubts about how to choose the most coherent and

well-balanced analytic strategy with respect to the parameters of time, cost and the results obtained.

The large majority of studies agree that there is a clear and close relationship between aneuploidies and male infertility. Moreover, they also agree that significant increases in the rate of aneuploidies usually correspond to percentages that are moderate in overall terms. However, the diagnostic interpretation of these increases and the genetic counseling that is subsequently given do not always coincide.

The present study has three objectives:

1. To identify those patients in which a FISH analysis of spermatozoa would be indicated.
2. To determine what chromosomes should be studied when seeking for effectiveness in terms of time, cost and obtained information.
3. To interpret the results in view to its clinical relevance.

In order to achieve these objectives we conducted a retrospective study of medical records and the results of FISH analyses of spermatozoa carried out in our laboratory between 1998 and 2005.

Materials and Methods

Study population

We analyzed semen samples from 319 individuals who consulted due to fertility problems and who were seen at six assisted reproduction centres. The mean age of patients was 36±5 years (range: 21 to 53). The control population was established in our laboratory and comprised six fertile and normozoospermic men who ranged in age from 20 to 25. All participants gave their informed consent with regard to participation in the study.

Criteria from the World Health Organization (10) were used to classify all the samples according to parameters of sperm count, motility and morphology. The reproductive history of the patients included the somatic karyotype in 266 men and the meiotic karyotype in 113; both results appear recorded in 98 cases.

Analysis of the somatic karyotype in peripheral blood was based on standard protocols and G-banded metaphase chromosomes. The meiotic preparations were obtained from testicular biopsy samples processed according to the protocol described by Evans *et al*. (11). Meiotic studies were based on the analysis of the different phases of spermatogenesis, thus enabling to assess meiotic arrest, synaptic abnormalities in metaphase I (desynapsis) and aneuploidies in metaphase II.

The characteristics of the patients according to semen parameters and to the somatic and meiotic karyotype are shown in Table 1.

Processing of semen samples

Semen samples were fixed in methanol:acetic acid (3:1) and spread onto microscope slides. Prior to applying the FISH protocol the nuclei of the spermatozoa were decondensed by incubating the preparations in 5 mM of dithiothreitol (DTT) and 1%

Triton X-100. The details of the fixation and decondensation protocols used have been previously described by Vidal *et al.*(12).

FISH protocol

Two hybridizations were performed for each sample: one used centromeric probes to study chromosomes X, Y, 18 (spectrum Green, spectrum Orange and spectrum Aqua, respectively), while the other employed specific locus probes for chromosomes 13 and 21 (spectrum Green and spectrum Orange) (AneuVysion Multicolor DNA Probe Kit; Vysis Inc., Downers Grove, IL, USA). In both cases the hybridization protocol applied was the standard one used in our laboratory (12).

Microscope evaluation

The preparations were evaluated under an Olympus BX60 fluorescence microscope equipped with a triple-band filter for DAPI/Texas Red/FITC and single-band filters for FITC, Texas Red and Aqua. A mean number of 508±131 spermatozoa were evaluated for each hybridization and patient in line with standardized analytic criteria (13). The control population was established from the analysis of 63,811 spermatozoa for chromosomes X, Y and 18 (10,635±438 spermatozoa per patient) and 62,345 spermatozoa for chromosomes 13 and 21 (10,390±192 spermatozoa per patient). The observed frequencies of disomy for the sex chromosomes and for chromosomes 13, 18 and 21, as well as the diploidy rates were recorded.

Statistical analysis

In order to determine whether the disomy and diploidy rates observed among infertile men were different to those in the control population we applied Fisher's exact test. This statistical comparison was performed for the five genotypes analyzed and the

148 results were considered in both population and individual terms, grouping the patients
149 according to the characteristics of the seminogram, the somatic karyotype and the
150 meiotic karyotype.
151 The correlation between an increased rate of chromosomal abnormalities and the
152 parameters of sperm count, motility, morphology and age was assessed by means of
153 Pearson's correlation coefficient.
154 All the analyses were performed using SPSS (version 13.0.1), with specialist assistance
155 being provided by the Statistics Service of the *Universitat Autònoma de Barcelona*. For
156 all statistical tests the level of significance was set at $p<0.05$.
157

158 **Results**

159 In the study population the rates of sex chromosome and chromosome 21 disomies were
160 higher than in the control group (0.41% vs. 0.19% and 0.15% vs. 0.07%, respectively;
161 $p<0.05$).

162 On the individual level, 49 of the 319 patients (15.36%) showed a significantly
163 increased rate of abnormalities for at least one of the chromosomes analyzed (Table 2).
164 The percentages that showed significant increases were moderate in overall terms,
165 ranging from 0.54 to 4.92% (mean: 1.41±1%). Disomy rates for chromosomes 13 and
166 18 were in all cases equivalent to those of the control population; this analysis excluded
167 the three individuals with a 45,XY,der(13;14)(q10;q10) karyotype.

168

169 When individuals were grouped according to the characteristics of the seminogram, all
170 of these groups included patients with an increased rate of chromosomal abnormalities
171 in their gametes (Table 3). The highest percentages were found in the groups of
172 oligozoospermic (2/4, 50%), oligoasthenozoospermic (17/51, 33.3%) and
173 oligoasthenoteratozoospermic (13/62, 21%) patients, the rate being between 5.9% and
174 15.4% in the remaining groups.

175 Of the three semen parameters analyzed (sperm count, motility and morphology) a
176 reduced sperm count was the only parameter that was correlated with the total
177 percentage of chromosome abnormalities ($R = -0.186$; $p=0.0009$) (Figure 1). As regards
178 the five genotypes analyzed a correlation was only observed for the rate of sex
179 chromosome disomy ($R=-0.158$; $p=0.005$) and diploidy ($R=-0.124$; $p=0.027$).

180

181 Among individuals with a normal somatic karyotype (245/266), 14% (34/245) had an
182 increased rate of chromosome abnormalities in the spermatozoa. In the group of
183 individuals with polymorphisms (13/266) a significant increase was observed in three of

the thirteen patients. In patients with karyotype abnormalities (8/266), we excluded from the statistical analysis the patient with a 47,XY,mar+ karyotype as the origin of the marker chromosome was unknown. In the remaining seven patients we only considered an increased rate of chromosome abnormalities when they did not affect the chromosomes involved in the alterations and, in this case, a significant increase was detected in one of the seven patients.

Among individuals with a normal meiotic result, 8.3% (3/36) had a significantly increased rate of chromosome abnormalities in their gametes, while the corresponding figure for patients with an abnormal meiotic karyotype was 26.5% (18/68) (Table 4).

There was no correlation between age and the percentage of chromosome abnormalities in spermatozoa ($R=0.1017$; $p=0.113$) (Figure 1). In this case, we only considered those men with a normal somatic karyotype. There was no significant difference between the mean age of the 34 men with a significantly increased rate of abnormalities (37±5 years; range 29-52) and the mean age of the remaining patients (35±5 years; range 21-52) ($p=0.088$; Mann Whitney U-test, $p<0.05$).

The individuals with a significantly increased rate of chromosome abnormalities were then grouped together in order to pool the data (see Table 2). It was found that 69% (34/49) of these patients had a low sperm count. Furthermore, 81% (34/42) of men with a known somatic karyotype had a normal karyotype. Among those patients in whom a meiotic study had been performed, 78% (18/23) presented meiotic abnormalities, mostly due to synaptic defects (57%, 13/23).

Discussion

Indications for a FISH analysis

It has been widely reported that the gametes of infertile individuals show a higher rate of chromosome abnormalities than the general population (14). The results from our series confirm once more this fact, but also illustrate the difficulty to delineate groups of patients having a clear indication for FISH studies.

- *Seminogram*

Several studies have shown a relationship between male infertility, semen quality and increased rates of aneuploidies in spermatozoa (8,9,15-19). However, it is difficult to assess the individual contribution of each of the three sperm parameters (count, motility and morphology) since the observed changes often appear in a combined way. Moreover, the great variability among the different published series hinders the comparison of results.

The relationship between the oligo condition and the rate of aneuploidies in spermatozoa has been analyzed in several studies. Like the present one, the majority of these reports show that infertile patients with a low sperm count often present higher rates of aneuploid spermatozoa (20-24). Furthermore, our analysis of the relationship between sperm count and the overall increased rates of chromosome abnormalities in spermatozoa revealed an inverse correlation between these two parameters, this being consistent with most of the series reported up to date (25 [50 patients]; 26 [15 patients]; 27 [19 patients]; 28 [30 patients]). It is well-know that during spermatogenesis various check-points are activated to arrest cells with chromosome abnormalities, leading to a reduction in gamete production and so to the oligo status (for a review, see 29). Inefficient control mechanisms could therefore be one explanation for the increased rate of chromosome abnormalities observed in spermatozoa. For example, there may be errors in the identification of abnormal cells, or a malfunction in the process of cell

elimination, or even that the number of abnormal cells was too high to be completely removed by the control systems.

Whereas the link between a low sperm count and an increased risk of aneuploidies in spermatozoa is widely accepted, the relationship between chromosome abnormalities and sperm motility is more controversial (7,30). Although some authors have reported a certain correlation between the two parameters (26,30-32), especially for sex chromosomes, other studies have failed to find any relationship (25,27.33). As regards our series, although some asthenozoospermic patients showed a significantly higher rate of chromosome abnormalities we found no correlation between these two parameters. Not even when analyzing the 67 patients purely asthenozoospermic ($R = -0.1218$; $p=0.326$).

Concerning sperm morphology, all studies that have analyzed individuals with polymorphic teratozoospermia report about some men with moderate, but significant increases, in the aneuploidy rate for the sex chromosomes (5,27,34-38). However, the results obtained in our series showed no correlation for this parameter, not even when analyzing the 17 exclusively teratozoospermic patients ($R=0.190016$; $p=0.469$); moreover, only one man presented a significant increase, in this case, for diploidy.

Thus, the relationship between an increased rate of chromosome abnormalities in spermatozoa and asthenozoospermia or teratozoospermia cannot be clearly established. To date, the isolated increases observed in asthenozoospermic, teratozoospermic and normozoospermic patients are of unknown aetiology.

- *Somatic karyotype*

Carriers of chromosome abnormalities often experience fertility problems as they have a certain risk of producing unbalanced spermatozoa for the chromosomes involved in the reorganization. Moreover, the reorganized chromosomes often show asynaptic regions

during the first meiotic division, which may interfere with the pairing and segregation of other unpaired segments (39). This phenomenon, known as *interchromosomal effect* (ICE) (40), has been suggested to occur in carriers of chromosomal abnormalities and also in individuals having polymorphic variants.

Although several studies have reported the occurrence of ICE in reorganization carriers (41-45) other authors have failed to replicate these findings (46,47). The population analyzed in our study included seven men with an altered somatic karyotype (Table 1). Their cytogenetic characteristics were very heterogeneous; therefore it was impossible to draw any conclusion about the potential benefits of sperm FISH analysis. With the aim of giving more relevance to these results we also took into account other studies published by us in infertile men carriers of chromosome reorganizations (Robertsonian translocations: (42); reciprocal translocations: (48,49); inversions: (50,51)). By pooling all these data, an interchromosomal effect can be noticed in 33% of men with Robertsonian translocations, in almost 44% of men with reciprocal translocations, and in 20% of men with inversions. These figures are clearly higher than the 14% infertile males showing abnormal FISH result, suggesting the participation of ICE as an additional source of numerical chromosome anomalies in the spermatozoa of reorganization carriers.

Regarding polymorphic variants, a common chromosome polymorphism in humans is the pericentric inversion of chromosome 9 (9qh). Although it is classified as a minor chromosomal reorganization, some studies have related these inversions to reproductive problems (52-54). In our study, one of the patients belonging to this group (1/9, 11%) showed a higher rate of chromosome abnormalities, specifically for diploidy, this being consistent with the report of Collodel *et al.*, (54). This rate is similar to the percentage found for the group of infertile individuals with a normal karyotype (11% versus 14%) suggesting no evidence for an ICE in these patients.

Heterochromatin polymorphisms are also considered a variant of the normal somatic karyotype, but they appear to be more common among infertile patients than in the general population (55-58). The only study to have analyzed chromosome abnormalities in the spermatozoa of these men described significant increases (58). Two out of the three patients with heterochromatin polymorphisms included in our study showed higher rates of chromosome abnormalities. Although we are aware that there is a need for larger studies to reach any conclusion, the results point to the production of sperm chromosome abnormalities in these individuals.

- *Meiotic karyotype*

Several studies have related errors in the processes of chromosome pairing and recombination with male infertility, either due to the production of aneuploid spermatozoa or to meiotic arrest (2,3,59).

In our population, and in line with previous findings, we observed two types of meiotic abnormalities: synaptic anomalies (81%) and meiotic arrest (16%), although in some cases both abnormalities were present (3%). Of the 55 individuals with synaptic abnormalities, only 21.8% showed a significantly increased rate of aneuploidy in their gametes. The corresponding percentages for patients with meiotic arrest and both abnormalities combined were 45.5% and 50% respectively. In all cases the incidence was clearly higher than that observed in patients with normal meiotic result (8%).

These results show that in 74% of patients there had been a clear reduction of abnormal cells across the process of spermatogenesis, probably due to the activation of checkpoints that would selectively eliminate aneuploid cells (60,61).

In any case, the percentage of patients with meiotic abnormalities who were carriers of chromosome abnormalities in their spermatozoa (26%) was also higher than the 14%

313 reported for infertile men with a normal karyotype suggesting, as in the case of somatic
314 karyotype, its implication in the generation of sperm aneuploidies.
315
316 - *Paternal age*
317 Some authors have sought to establish a relationship between the presence of
318 chromosome abnormalities in gametes and specific factors such as age of the parents.
319 Although it is known that advanced maternal age is a risk factor for giving birth to
320 children with aneuploidies, there are rather mixed results for the risk associated with
321 advanced paternal age (62-65).
322 Not too much research has been devoted to analyze this relationship in infertile men.
323 While some studies found no correlation (66,67) other authors report an effect-age, but
324 exclusively on the rate of sex chromosome disomy (65,68). Furthermore, it should be
325 mentioned that Plastira *et al.* (65) found a higher rate of sex chromosome aneuploidy in
326 younger men.
327 Our study showed no correlation for any of the chromosome abnormalities analyzed.
328 Moreover, we found no differences between the age of patients showing a significantly
329 increased rate (mean 37±5; range 29-52) and the age of the remainder (mean 35±5;
330 range 21-52). In view of these results it can be considered that above a certain age (52
331 years; the maximum age in our series), the rate of chromosome abnormalities could
332 increase; however, a recent study of almost 100 individuals ranging in age from 22 to
333 80 also failed to show any age effect (64).
334
335 In summary, and to answer the first objective of this study, a FISH analysis of
336 spermatozoa is especially indicated in:

1. Individuals with a normal somatic karyotype and low sperm count. For other abnormalities in the seminogram its application will depend on the couple's reproductive history.

2. Individuals with an abnormal somatic karyotype or who are carriers of heterochromatic polymorphisms.

3. Individuals with an abnormal meiotic karyotype.

Chromosomes to be studied

To attain the objective of determining the chromosomes to be studied to optimize the technique in terms of time, cost and information, we conducted an exhaustive review of the literature that has reported a significantly increased rate of aneuploidies in infertile men. Taken as a whole, these studies have evaluated chromosomes 1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 21, 22, X and Y (15,18-20,22-28,30-33,35,36,69-80). Almost all papers have evaluated the anomalies for the sex-chromosomes plus one or more autosome. Reviewing the results individual by individual, only four patients showed an increase in the rate of abnormalities for autosomes alone (chromosome 21 disomy (18,70,78); chromosome 18 disomy (18); chromosome 15 disomy (75)).

In our series we found a significantly increased rate of sex chromosome disomies and chromosome 21 disomy. In contrast, the disomy rate in chromosomes 13 and 18 was in all cases equivalent to that found in the control population. These finding are consistent with previous studies reporting that the sex chromosomes and chromosome 21 show higher percentages of non-disjunction when compared with other chromosomes (13,59,81). Furthermore, compiling all the results reported up to date, it can be stated that the presence of increased rates of abnormalities affecting only autosomes, with the exception of chromosome 21, seldom occur.

Thus, regarding the second objective of our study, we can conclude that the study of chromosomes 21, X and Y would be enough to identify the large majority of patients at risk, that is, those individuals with a higher probability of producing chromosomally abnormal spermatozoa than is found among the general population. Indeed, Ferguson *et al.* (82) have already suggested that the analysis of recombination events between sex chromosomes could be a useful indicator for identifying men with an increased risk of producing chromosomally abnormal spermatozoa.

Interpreting the FISH results

The increases in the anomalies are in line with percentages described in most of the studies reviewed in the present report. Furthermore, it is evident that the raw numbers are moderate in overall terms (ranging from 0.54% to 4.92%; mean 1.41±1%). Accordingly, the clinical relevance of the sperm-FISH results merits to be addressed.

Abnormal results in sperm-FISH analyses could be considered from two different perspectives:

- Quantitative: the significant increases would be interpreted as a numerical value that would indicate the patient's degree of risk. The main drawback of this interpretation derives from the characteristics of the technique itself. Firstly, it is very difficult to analyze all the chromosomes of the karyotype (normally only X, Y, 13, 18 and 21 are analyzed), and secondly not all the chromosome abnormalities are evaluated (e.g. nullisomies are not considered). Moreover, the assessment criteria used are very strict and yield an estimate on the low side of real rates; therefore, in our view it would be a mistake to give them a strictly numerical interpretation.

- Qualitative: in this case the significant increases would have to be interpreted as evidence that there are abnormalities in the pairing, recombination and/or segregation of meiotic chromosomes, thus indicating that the quality of the spermatogenesis is not

optimum. This interpretation supports the proposal that the study of only three chromosomes (21, X and Y), and the analysis of a specific type of abnormality, would be sufficient to identify errors in the meiotic process and, therefore, to identify the large majority of at-risk patients. In fact, the value of the qualitative interpretation compared to the quantitative approach is supported by several pieces of data. For example, it would be difficult to explain by means of a strictly quantitative analysis the clinical repercussions of chromosome abnormalities in spermatozoa on IVF/ICSI cycles. Aneuploidies in these gametes have been associated with implantation errors (83,84), recurrent miscarriages (33,71) and also with chromosome anomalies in live births (85,86).

Therefore, and as regards the third objective, FISH analysis of spermatozoa should be used as a tool of genetic screening in infertile patients). Significant differences in the rates of chromosome abnormalities with respect to controls should be taken into consideration regardless the numerical value. When abnormal results are obtained, individuals should be identified as “at risk” and the couple should be advised about the available techniques of preimplantation and prenatal genetic diagnosis.

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744 **Table 1.**

Categories of classification		No. patients	% patients	Age range (years)	
Seminal parameters					
Asthenoteratozoospermia (AT)		71	22.3	27-53	
Asthenozoospermia (A)		67	21.0	27-49	
Normozoospermia (N)		34	10.7	28-52	
Oligoasthenoteratozoospermia (OAT)		62	19.4	21-46	
Oligoasthenozoospermia (OA)		51	16.0	28-48	
Oligoteratozoospermia (OT)		13	4.1	26-41	
Oligozoospermia (O)		4	1.3	33-37	
Teratozoospermia (T)		17	5.2	30-52	
	Total	**319**	**100.0**	**21-53**	
Karyotype					**Detailed Karyotype**
46,XY		245	92.1	21-52	
					46,XY,1qh+
					46,XY,inv9(p11q12)
					46,XY,inv9(p11q12)
					46,XY,inv9(p11q12)
					46,XY,inv9(p11q12)
					46,XY,inv9(p11q13)
Polymorphisms		13	4.9	29-45	46,XY,inv9(p11q12)
					46,XY,inv9(p11q12)
					46,XY,inv9(p11q12)
					46,XY,inv9(p11q12)
					46.XY,16qht
					46,XY,22s+
					46,X,invY(p11.1q11.2)
					46,XY,t(3;16)(p21;q13)
					46,XY,t(5;19)(12;p13.3)
					45,XY,der(13;14)(q10;q10)
					45,XY,der(13;14)(q10;q10)
Abnormal		8	3.0	27-40	45,XY,der(13;14)(q10;q10)
					46,X,invY(p11.1q11.23),inv4(p14p15.3)
					47,XXY(50%)/46,XY(50%)
					47,XY,mar+
	Total	**266**	**100.0**	**21-52**	
Meiotic study					
Normal		36	31.9	27-49	
Abnormal	Desynapsis	55	48.7	26-52	
	Arrest	11	9.7	29-44	
	Desynapsis and arrest	2	1.8	33,34	
Non informative		9	7.9	27-42	
	Total	**113**	**100.0**	**26-52**	

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747 **Table 2.**

Patient code	Seminal parameters	Age	Karyotype	Meiotic study	Disomy (%)				Diplody (%)
					Chr. 13	Chr.18	Chr.21	Chr. XY	
180	AT	52	46,XY	Abnormal (D)				2.82	
70	AT	36	46,XY	Normal					0.59
118	AT	45	46,XY	Abnormal (D)					0.79
64	AT	45	46,XY,inv9(p11q12)	Abnormal (D)					1.08
140	AT	39	unknown	-					0.59
99	AT	33	unknown	-				1.00	
165	A	36	46,XY	-			0.96		
184	A	37	46,XY	Abnormal (D)			1.37		0.68
16	A	33	46,XY	-				0.93	
42	A	30	46,XY	-					2.67
193	N	39	46,XY	Normal					0.56
24	N	29	46,XY	-				0.79	
286	N	42	46,XY	Abnormal (D)				1.93	
388	N	34	46,XY	-					1.97
336	OAT	29	46,XY	Abnormal (A)					0.57
191	OAT	37	46,XY	-					0.78
33	OAT	39	46,XY	-				0.96	
129	OAT	37	46,XY	-				0.98	
112	OAT	45	46,XY	Abnormal (D)				3.15	
59	OAT	36	46,XY	Abnormal (D)				2.25	0.90
39	OAT	33	46,XY	-				0.78	0.71
178	OAT	32	46,XY	Normal				1.15	1.76
86	OAT	29	46,XY,1qh+	Abnormal (D)				1.14	1.27
51	OAT	34	45,XY,der(13;14)(q10;q10)	Abnormal (A)	1.79				
233	OAT	36	unknown	-			0.88		
199	OAT	29	unknown	Abnormal (A)			1.00	3.27	0.91
17	OAT	29	unknown	Abnormal (D)			1.10		0.76
26	OA	35	46,XY	-			0.97		
215	OA	37	46,XY	-				4.49	0.79
217	OA	38	46,XY	-					0.54
232	OA	48	46,XY	-					0.68
242	OA	39	46,XY	Abnormal (D)				1.54	
166	OA	36	46,XY	Abnormal (D)					1.26
12	OA	31	46,XY	-				1.03	0.87
243	OA	38	46,XY	Abnormal (D)				1.84	
287	OA	40	46,XY	-				4.92	
398	OA	33	46,XY	Non informative				1.82	1.40
399	OA	34	46,XY	-					0.70
172	OA	38	46,XY	Abnormal (A)					0.59
328	OA	34	46,XY,22s+	Abnormal (DA)				2.40	
264	OA	32	46,XY,(3;16)(p21;q13)	-					2.71
331	OA	39	47,XXY (50%)/46,XY(50%)	-			1.99		
356	OA	40	45,XY,der(13;14)(q10;q10)	-	0.59				0.98
48	OA	37	unknown	-				0.78	
188	OT	34	46,XY	Abnormal (A)					0.77
376	OT	27	45,XY,der(13;14)(q10;q10)	-	4.30				
279	O	35	46,XY	-				1.37	0.77
18	O	37	unknown	Non informative				1.61	
355	T	37	46,XY	-					0.96
Controls	N	20-25	46,XY	-	0.06	0.03	0.07	0.19	0.22

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750 **Table 3.**
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Seminal parameters	Altered FISH results	%
asthenoteratozoospermic (AT)	6/71	8.5
asthenozoospermic (A)	4/67	6.0
normozoospermic (N)	4/34	11.8
oligoasthenoteratozoospermic (OAT)	13/62	21.0
oligoasthenozoospermic (OA)	17/51	33.3
oligoteratozoospermic (OT)	2/13	15.4
oligozoospermic (O)	2/4	50.0
teratozoospermic (T)	1/17	5.9
Total	**49/319**	**15.36**

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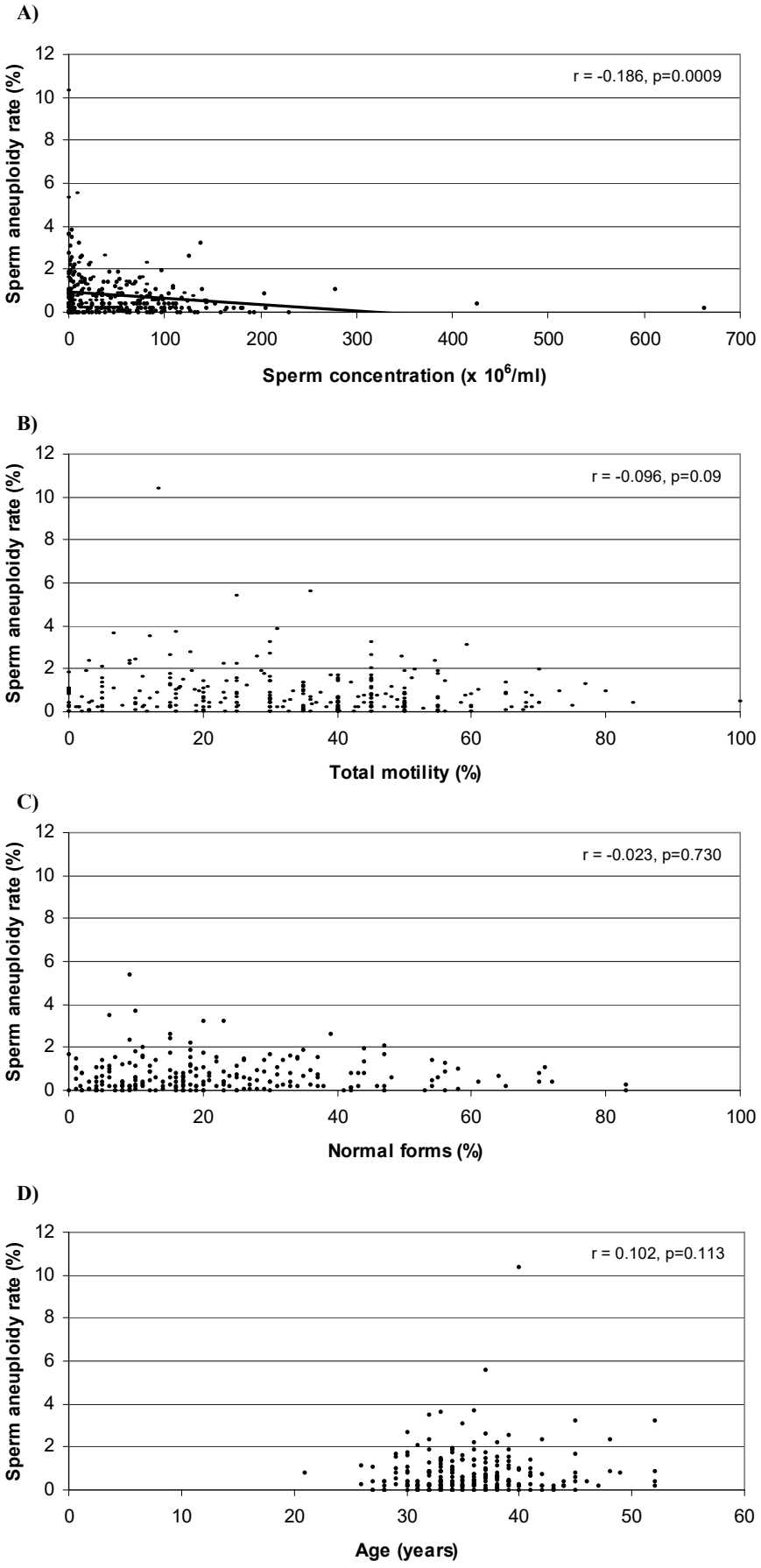
Figure 1.


A)
r = -0.186, p=0.0009
Sperm aneuploidy rate (%)
Sperm concentration (x 10^6/ml)
B)
r = -0.096, p=0.09
Sperm aneuploidy rate (%)
Total motility (%)
C)
r = -0.023, p=0.730
Sperm aneuploidy rate (%)
Normal forms (%)
D)
r = 0.102, p=0.113
Sperm aneuploidy rate (%)
Age (years)

802 **Table 4.**
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Meiotic study		**Altered FISH results**	**%**
Normal		3/36	8.3
Abnormal	Desynapsis (D)	12/55	21.8
	Arrest (A)	5/11	45.5
	Desynapsis and arrest (DA)	1/2	50.0
Non informative		2/9	22.2
Total		**23/113**	**20.4**

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Legends

Table 1. Patients classification according to seminal parameters, somatic karyotype and meiotic study

Table 2. Characteristics of patients with increased aneuploidies rates in their gametes ($p \leq 0.05$)

D: desynapsis; A: arrest; DA: desynapsis and arrest

Table 3. Patients with abnormal FISH results classified according to their seminal parameters

Figure 1. Scatter plot of sperm concentration (**A**), total motility (**B**), percentage of normal forms (**C**) and age (**D**) versus the total sperm aneuploidy rate.

Table 4. Patients with chromosomal abnormalities in spermatozoa classified according to the meiotic study results.