Impact of different patterns of sperm chromosomal abnormalities on the chromosomal constitution of preimplantation embryos

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Objective: To evaluate the effect of sperm chromosome abnormalities—disomy for sex chromosomes and diploidy—in the chromosomal constitution of preimplantation embryos.
Design: Retrospective cohort study.
Setting: Infertility clinic.
Patient(s): Three groups: 46,XY infertile men with increased incidence of sex chromosome disomy in sperm; 46,XY infertile men with increased diploidy rates in sperm; 47,XYY infertile men with increased sex chromosome disomy and diploidy rates in sperm.
Intervention(s): Sperm collection for fluorescence in situ hybridization analysis. Embryo biopsy for preimplantation genetic screening.
Main Outcome Measure(s): Frequencies of numerical abnormalities in sperm for chromosomes 13, 18, 21, X, and Y, and in embryos for chromosomes 13, 16, 18, 21, 22, X, and Y.
Result(s): A significant increase of chromosomally abnormal and mosaic embryos was observed in the three study groups compared with controls. Those sperm samples with increased sex chromosome disomy rates produced significantly higher percentages of aneuploid embryos, with a threefold increase for sex chromosomes. Sperm samples with increased diploidy rates were mainly associated to the production of triploid embryos.
Conclusion(s): A strong correlation between sperm and embryo chromosomal constitution has been shown in infertile men with 46,XY and 47,XYY karyotypes. (Fertil Steril® 2010;94:1380–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: FISH, PGS, chromosomal abnormalities, spermatozoa, embryos

In recent years intracytoplasmic sperm injection (ICSI) has improved the chances of achieving pregnancy of couples with severe male factor infertility. Prenatal testing in ICSI pregnancies has shown 2.1% of de novo chromosome abnormalities in men with less than 20×10^6 sperm/mL, with an incidence of 0.6% for sex chromosomes (1). These elevated rates have been associated more with the sperm quality than with the ICSI procedure itself (2).

In fact, fluorescence in situ hybridization (FISH) analysis of sperm from normal karyotype infertile men has shown increased levels of aneuploid and diploid spermatozoa in which the sex chromosomes are mainly affected. This increase is higher in severe oligoasthenoteratozoospermic (OAT) men with less than 5×10^6 sperm/mL (3–6) and in azoospermic men (7–9). In sex chromosome aneuploidies, the pachytene checkpoint mechanism produces a complete or partial meiotic arrest of the abnormal cells that suffer nondisjunction of sex chromosome bivalent during meiosis I or II. Occasionally, mutations of one or more of the genes involved in these DNA repair mechanisms produce chromosomally abnormal cells that escape the pachytene checkpoint and result in spermatozoa with disomy for sex chromosomes. A delay in synapsis or the existence of heterosynapses between unpaired regions of some chromosomes could generate an inappropriate alignment on the metaphase plate, with chromosomes being unable to migrate to the poles at anaphase I (10). If there is a lack in the anaphase I checkpoint that arrest the meiotic process, the cell does not divide and produces a single diploid secondary spermatocyte, giving rise to two diploid spermatozoa after meiosis II (reviewed in Refs. 11, 12).

In the literature a variable meiotic behavior is described among 47,XYY men. Whereas some investigators report a total absence of abnormal sperm (12, 13), others have described an increase of diploidy (14) or an increase of both sex chromosome aneuploidy and diploidy (15–21). The FISH studies and immunofluorescence techniques have detected the presence of XY and XYY pachytene cells together in 47,XYY men (10). Analysis of different stages of gametogenesis suggests that the pachytene I checkpoint produces meiotic arrest of XYY cells, leading to oligozoospermia or azoospermia (12, 19, 22). However, other meiotic studies have shown that a small number of XYY premeiotic cells can escape the

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pachytene checkpoint, achieve meiosis, and produce chromosomally abnormal spermatozoa (11, 12, 20, 23). In addition, it has been proposed that sperm count and aneuploid sperm production in 47,XYY men is directly dependent of the XYY pachytene cells proportion (10).

The presence of chromosomally abnormal sperm has been related to recurrent miscarriage (4, 24–26) and, more recently, with repetitive ICSI failures (27, 28). Preimplantation genetic diagnosis for aneuploidy screening (PGS) has been proposed as a tool for detecting possible chromosomal abnormalities in embryos before their replacement to the uterus. The application of PGS in couples with a high incidence of sperm chromosome abnormalities or a 47,XYY karyotype has revealed a high incidence of chromosomally abnormal embryos (21, 29) and, consequently, allows their reproductive outcome to improve (30).

In this retrospective study we have evaluated sperm chromosome abnormalities in 46,XY and 47,XYY infertile men and their implications in the chromosomal constitution of day 3 embryos.

MATERIALS AND METHODS

Patients

This is a retrospective study carried out from July 1999 to December 2007, in which 80 PGS cycles were performed in 60 couples with severe male factor infertility: patients with 46,XY karyotype and increased incidence of sperm sex chromosome disomy ($n = 37$) or diploidy ($n = 18$), and pure or mosaic 47,XYY patients ($n = 5$). The study was approved by the Institutional Review Board (IRB) of the Instituto Valenciano de Infertilidad. Chromosomal abnormalities in spermatozoa were analyzed by FISH and sperm samples were classified as abnormal when the number of spermatozoa with abnormalities for at least one chromosome was significantly higher than that observed in a control group of 14 normozoospermic donors (9, 15, 31).

To assess the impact of sperm chromosomal abnormalities on preimplantation embryos, three groups of patients were considered.

Group 1 Forty-six PGS cycles were performed in patients with normal karyotype and abnormal FISH sperm results due to an isolated increase of sex chromosome disomy compared with the control group of normozoospermic donors (see Fig. 1 for description of disomy and diploidy rates for chromosomes 13, 18, 21, X, and Y in group 1 men and controls). Two of the patients had obstructive azoospermia, and spermatozoa were retrieved from the testicle. The remaining 35 samples were ejaculated sperm. Mean male age was 35.0 years (range 27–42 years), mean sperm concentration was 1.0×10^6 sperm/mL (range $0.1–37.0 \times 10^6$ sperm/mL), mean sperm motility was 21.0% (range 1%–58%), and mean percentage of sperm with normal morphology was 1.5% (range 0–8%) (32).

Group 2 Twenty-seven PGS cycles were performed in patients with normal karyotype and abnormal FISH sperm results due to an isolated increase of diploid sperm compared with the control group (see Fig. 1 for description of disomy and diploidy rates for chromosomes 13, 18, 21, X, and Y in group 2 men and controls). All samples were ejaculated sperm. Mean male age was 35.0 years (range 31–38 years), mean sperm concentration was 23.5×10^6 sperm/mL (range $0.1–106.0 \times 10^6$ sperm/mL), mean sperm motility was 46.0% (range 3%–69%), and mean percentage of sperm with normal morphology was 1.0% (range 0–8%). No previous history of recurrent miscarriage or implantation failure was recorded in any of the couples in group 1 or group 2.

Group 3 Seven PGS cycles were performed in four patients with 47,XYY karyotype and one patient with a 47,XYY/46,XY karyotype. In four PGS cycles (2 couples), FISH analysis in sperm showed a significant increase of disomy for sex chromosomes or diploidy compared with the control group, and in the remaining three PGS cycles (3 couples) no increase of sperm chromosomal abnormalities were observed after FISH analysis (Table 1). One 47,XYY patient had obstructive azoospermia and spermatozoa were retrieved from the testis, whereas the remaining samples were ejaculated sperm. Mean male age was 33.2 years (range 30–36 years), mean sperm concentration was 23.1×10^6 sperm/mL (range $0.6–69.0 \times 10^6$ sperm/mL), mean sperm motility was 48.5% (range 42%–55%), and mean percentage of sperm with normal morphology was 5.3% (range 0–12%) (data from ejaculated samples).

For statistical comparisons, a control group of 28 fertile couples with normal karyotypes who underwent PGS for sex-linked diseases ($n = 33$ cycles) was included in the study. All male partners were normozoospermic and sperm FISH studies were not indicated in this group. Female age in all PGS cycles (study and control groups) was ≤ 37 years.

FISH Sperm Studies

Ejaculated or testicular sperm samples were prepared for FISH analysis as previously described (4, 9). Sperm nuclei were decondensed by slide incubation for 5–7 minutes at 37°C in 5 mmol/L dithiothreitol (DTT) and 1% Triton X-100. DNA was denatured for 5 minutes at $73° \pm 1°$C in a water bath in 70% formamide. Numerical abnormalities for chromosomes 13, 18, 21, X, and Y were evaluated in different slides from the same sample (33). Centromeric DNA probes for chromosome 18 (locus D18Z1, CEP 18 Spectrum Aqua; Vysis Inc., Downers Grove, IL), chromosome X (locus DXZ1, CEP X Spectrum Green; Vysis Inc.), and chromosome Y (locus DYZ3, CEP Y Spectrum Orange; Vysis Inc.) were used for triple-color FISH analysis. Locus-specific DNA probes for chromosome 13 (locus RB, LSI 13 Spectrum Green; Vysis Inc.) and chromosome 21 (loci D21S259, D21S341, D21S342, LSI 21 Spectrum Orange; Vysis Inc.) were used for dual-color FISH analysis. The FISH incubation and detection were performed according to the manufacturer's instructions.

Analysis was carried out using an Olympus AX70 epifluorescence microscope equipped with a triple-band pass filter for 4'6-diamidino-2-phenylindole (DAPI)/Texas-Red/ fluorescein isothiocyanate conjugate (FITC) and single-band pass filters for FITC, Texas Red, and Aqua Blue (Olympus España, S.A.U.). The number of spermatozoa per chromosome evaluated in each sample varied according to the sperm concentration, ranging from 286–7,302 sperm cells. Spermatozoa with disomy and diploidy for the analyzed chromosomes were scored as abnormal. Nullisomic spermatozoa were not directly assessed due to the difficulty of differentiating them from hybridization failure.

Preimplantation Genetic Screening

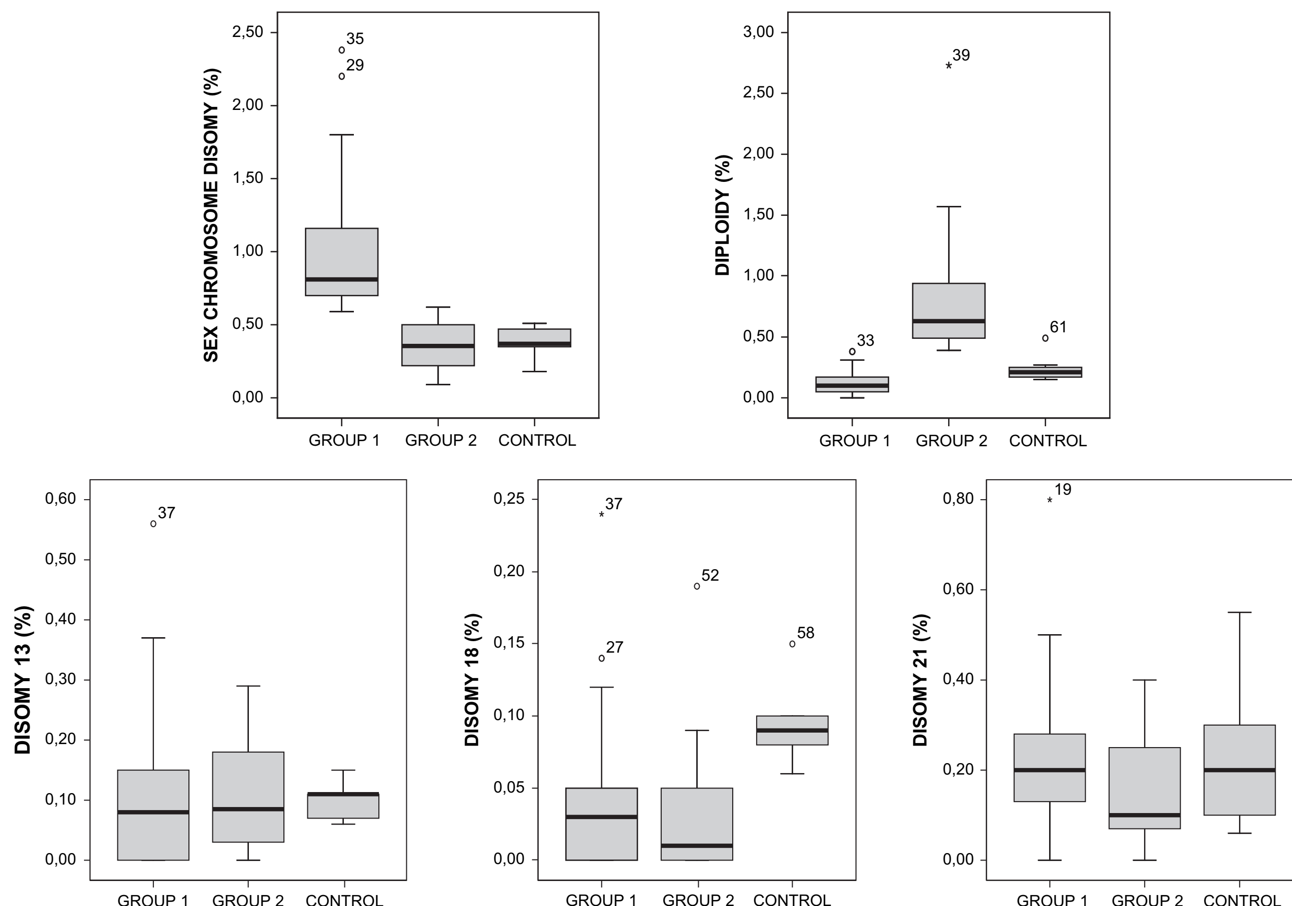
After ovarian stimulation, oocyte retrieval was carried out by transvaginal aspiration of ovaries under ultrasound guidance. The ICSI was performed in all the cycles; fertilization was assessed 17–20 hours after ICSI (day 1) and embryo cleavage 24 hours thereafter (day 2). Embryo biopsy was performed on day 3 embryos with ≥ 5 nucleated blastomeres and $\leq 25\%$ fragmentation degree. One or two blastomeres were withdrawn depending on the number of cells. For the biopsy, embryos were placed on a droplet containing Ca^{2+}- and Mg^{2+}-free medium (G-PGD; Vitrolife, Göteborg, Sweden), and Tyrode's solution (Vitrolife) or laser technology (OCTAX, Herbron, Germany) was used to perforate the zona pellucida (ZP) (30, 34). After biopsy, embryos were washed and cocultured on a monolayer of endometrial epithelial cells (35). Individual blastomeres were fixed under an inverted microscope using a modified Tarkowski's protocol without hypotonic pretreatment (36). The FISH protocol used in our laboratory for aneuploidy screening was simplified during the course of the study from three to two rounds of hybridization (37). In the two-rounds protocol, the first round included locus-specific or centromeric probes for chromosomes 13, 16, 18, 21, and 22 (Multivysion PB; Vysis Inc.), and the second round used centromeric probes for chromosomes X and Y. Starting in 2004, chromosome 15 was also analyzed in the second round. Because chromosome 15 was not evaluated in all the PGS cycles performed in this study, it was not included in the individualized analysis of chromosomal abnormalities. Detection washings and signal scoring were carried out following manufacturer's instructions. The FISH analysis was performed using the same epifluorescent microscope as previously described and including single-band pass filters for Spectrum Gold and Blue.

Statistical Analysis

For FISH sperm studies, the percentages of diploid and disomic spermatozoa for each chromosome were scored. For FISH analysis of blastomeres,

FIGURE 1

Descriptive values of FISH in sperm for chromosomes 13, 18, 21, X, and Y in infertile 46,XY patients. Group 1 represents patients with significantly increased sex chromosomes disomy rates compared with controls. Group 2 represents patients with significantly increased diploidy rates compared with controls. Atypical (°) and extreme (*) values observed for disomy 13, 18, and 21 did not show statistical differences with controls.



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percentages of numerical chromosomal abnormalities (aneuploidy and triploidy) and mosaicism (defined as discordant results when two blastomeres from the same embryo were analyzed) were evaluated. The χ^2 test and the Fisher's exact test with Bonferroni's correction for multiple group comparisons were used for statistical analysis. Level of significance was selected as $P<.05$.

RESULTS

FISH Sperm Studies

In group 1, the incidence of sex chromosome disomy was almost threefold increased compared with controls (mean of 0.89% vs. 0.37%, $P<.001$), mainly due to the presence of 24,XY spermatozoa (mean of 0.69% vs. 0.11%, $P<.001$). Group 2 showed a threefold increase of diploid sperm compared with controls (mean of 0.64% vs. 0.25%, $P<.001$) (Table 2). And, two patients with 47,XYY karyotype (group 3) showed significantly higher sex chromosome disomy rates ($P<.05$) or diploidy rates ($P<.0001$) compared with controls (Table 1). In group 3, sex chromosome abnormalities were due to the presence of 24,XY and 24,YY spermatozoa (mean of 0.29% and 0.28%, respectively).

Preimplantation Genetic Screening

Table 2 shows the correlation of chromosomal abnormalities between sperm and embryos. The three study groups displayed high percentages of chromosomally abnormal and mosaic embryos, all of them significantly increased compared with the PGS control group. Embryo aneuploidy for each individual chromosome and the percentage of triploid embryos were also evaluated. Interestingly, in patients with an isolated increase of sperm sex chromosome disomy (group 1), there was a significantly higher incidence of aneuploid embryos in which not only the sex chromosomes but also all the analyzed autosomes were affected ($P<.05$ vs. control group). This group did not show statistical differences in triploid embryos. On the other hand, in the case of patients with an isolated increase of diploid sperm (group 2), there was a significant increase of triploid embryos compared with controls ($P<.05$) and a significantly higher rate of embryos with aneuploidy for only chromosomes 16 and 22. Finally, patients with 47,XYY karyotype (group 3) presented a remarkably high incidence of triploid embryos compared with controls and a significant increase of embryo aneuploidies only for sex chromosomes ($P<.05$).

TABLE 1

Percentage of sperm chromosomal abnormalities in 47,XYY patients.

Patient	Karyotype	Scored XY18	Sex chromosome disomy (%)	Disomy 18 (%)	Scored 13/21	Disomy 13 (%)	Disomy 21 (%)	Diploidy (%)	FISH result
1	47,XYY	2,194	0.77[c]	0.05	2,016	0.15	0.15	0.10	Abnormal
2	47,XYY	5,338	0.56[b]	0.11	—	—	—	0.82[d]	Abnormal
3	47,XYY/46,XY	2,759	0.36	0.11	2,021	0.05	0.15	0.10	Normal
4	47,XYY	2,143	0.09	0	2,035	0	0.10	0.38	Normal
5	47,XYY	2,553	0.35	0	2,025	0	0.20	0.02	Normal
Controls[a]	46,XY	50,572	0.37 ± 0.13	0.10 ± 0.03	70,086	0.10 ± 0.04	0.38 ± 0.12	0.25 ± 0.10	—

[a] Data correspond to mean ±SD of the 14 donors.
[b] $P < .05$.
[c] $P < .01$.
[d] $P < .0001$ vs. control group; χ^2 test.

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Although similar rates of chromosomally abnormal embryos according to their FISH sperm results were observed in group 3, embryo aneuploidy for sex chromosomes was higher in patients with abnormal than in patients with normal FISH sperm results (25.0% and 16.7%, respectively).

After a first PGS cycle, at least one subsequent PGS cycle was performed in seven couples from group 1, in six couples from group 2, and in three couples from group 3. Similar results were observed among the first and the subsequent PGS cycles in the percentage of chromosomally abnormal embryos (64.3% vs. 70.2% in group 1; 64.7% vs. 56.6% in group 2; and 47.1% vs. 64.7% in group 3), embryo aneuploidy for sex chromosomes (14.0% vs. 20.9% in group 1; 5.9% vs. 11.3% in group 2; and 23.5% vs. 17.6% in group 3), and triploid embryos (0% vs.

TABLE 2

Comparisons of sperm and embryo chromosomal abnormalities in the three study groups and controls.

	Group 1 (37 couples)		Group 2 (18 couples)		Group 3 (5 couples)		Controls (14 donors for sperm; 28 couples for PGS)	
	Sperm	Embryos	Sperm	Embryos	Sperm	Embryos	Sperm	Embryos
No. of sperm/embryo analyzed	126,178	226	96,422	136	23,084	36	120,658	200
No. of PGS cycles	—	46	—	27	—	7	—	33
Abnormal embryos (%)	—	141 (62.38)[c]	—	80 (58.82)[c]	—	21 (58.33)[b]	—	68 (34.0)
Mosaic embryos (%)	—	46 (41.81)[c]	—	23 (29.87)[b]	—	4 (40.00)[b]	—	7 (7.4)
% Chromosome 13 abnormalities[d]	0.08	16.22[b]	0.10	12.12	0.05	13.89	0.10	5.24
% Chromosome 16 abnormalities[d]	—	23.36[c]	—	19.85[a]	—	19.44	—	8.57
% Chromosome 18 abnormalities[d]	0.04	18.10[b]	0.03	11.19	0.07	11.11	0.10	7.07
% Chromosome 21 abnormalities[d]	0.20	21.93[b]	0.14	12.78	0.15	5.56	0.38	9.68
% Chromosome 22 abnormalities[d]	—	21.72[c]	—	17.91[a]	—	11.11	—	7.47
% Chromosome XY abnormalities[d]	0.89[c]	20.64[c]	0.35	10.37	0.45	22.22[a]	0.37	7.54
% Ploidy abnormalities[e]	0.10	0.88	0.64[c]	3.67[a]	0.32	5.56	0.25	0

Note: The percentage of mosaic embryos was calculated as follows: No. of embryos with two cells showing discordant results / No. of embryos with two cells analysed. PGS = preimplantation genetic screening.
[a] $P < .05$.
[b] $P < .01$.
[c] $P < .001$; vs. controls; χ^2 test and Fisher exact test with Bonferroni's correction.
[d] Disomy for sperm, aneuploidy for embryos.
[e] Diploidy for sperm, triploidy for embryos.

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TABLE 3

Potentially viable chromosomal abnormalities diagnosed in preimplantation embryos.

	GROUP 1 (226 embryos)	GROUP 2 (136 embryos)	GROUP 3 (36 embryos)	PGS CONTROL (200 embryos)
Trisomy 13 (%)	2 (0.9)	4 (2.9)	–	1 (0.5)
Trisomy 18 (%)	1 (0.4)	1 (0.7)	1 (2.8)	2 (1.0)
Trisomy 21 (%)	6 (2.7)	4 (2.9)	–	4 (2.0)
Sex chromosome abnormalities (%)	14 (6.2)	1 (0.7)	3 (8.3)	4 (2.0)
Monosomy X	4	1	1	1
Trisomy XXX	4	–	1	–
Trisomy XXY	2	–	–	1
Trisomy XYY	4	–	–	2
Tetrasomy XXYY	–	–	1	–
Composed abnormalities (%)	1 (0.4)	1 (0.7)	–	1 (0.5)
Mosaic trisomy 13 and 18/2n	1	–	–	–
Mosaic monosomy X/trisomy XXX	–	1	–	–
Trisomy 21+trisomy XXX	–	–	–	1
Total viable abnormalities (%)	24 (10.6)	11 (8.1)	4 (11.1)	12 (6.0)

Notes: NS vs. control ($p<0.05$; Fisher exact test with Bonferroni's correction).

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2.1% in group 1; 5.9% vs. 3.8% in group 2; and 0% vs. 11.8% in group 3).

Table 3 reflects the incidence of potentially viable chromosomal abnormalities (abnormalities compatible with life) diagnosed in preimplantation embryos. Groups 1 and 3 showed a threefold and a fourfold increase in the percentages of potentially viable embryos with sex chromosome aneuploidies compared with controls (6.2% and 8.3% vs. 2.0%, respectively). All potentially viable chromosomal abnormalities in group 3 were from patients with abnormal FISH sperm results, with a total percentage of viable chromosomal abnormalities of 16.7% (4/24 embryos) in this subgroup, specifically 12.5% for sex chromosomes. Similar incidences of potentially abnormal viable embryos with trisomy for chromosomes 13, 18, 21, or complex abnormalities were observed in all groups.

DISCUSSION

Our study shows that a significant increase in sperm chromosome abnormalities has a direct effect on the chromosomal constitution of preimplantation embryos. The genetic risk seems to vary according to the type of sperm chromosomal abnormality detected. Whereas an increase of disomy for sex chromosomes generates elevated rates of potentially viable embryos whose sex chromosomes are affected, increases of diploid sperm are associated with abnormalities that end in miscarriages. In addition, in 47,XYY patients in which FISH in sperm showed increases of both sex chromosomes disomy and diploidy, the chromosomal constitution observed in their embryos seems to be the cumulative effect of the two patterns of sperm chromosomal abnormalities, with increases of aneuploid embryos and a high incidence of triploid embryos.

In this study, normal karyotype patients with sex chromosome abnormalities in their spermatozoa presented meiotic errors mainly in meiosis I, producing a high proportion of 24,XY spermatozoa. These results are in accordance with those of most FISH studies of sperm from infertile men (5, 8, 25, 38, 39). In 47,XYY patients, a high incidence of 24,XY and 24,YY sperm was observed, which are also representative of meiotic I errors. These findings concur with those of previous reports (10, 15, 17, 19, 20). However, some investigators have also described higher rates of 24,XX sperm in 47,XYY infertile patients (10, 21, 40), which we did not observe.

An inverse correlation between sperm parameters (mainly sperm count) and meiotic errors in infertile patients with both normal and abnormal karyotypes has been reported (4, 5, 21, 41). Interestingly, most of our study subjects with normal karyotype and isolated sex chromosome abnormalities were oligozoospermic, and most patients with isolated diploidy had normal sperm counts. This could be a reflection of the different meiotic origins of sex chromosome disomy and diploidy (10–12). The presence of abnormal sex chromosome bivalents seems to be detrimental to cell progression, as most of them are eliminated at the pachytene checkpoint, which results in oligozoospermia or azoospermia. On the other hand, the presence of a complete double set of chromosomes does not seem to be so detrimental, as the cell is able to continue meiosis, which leads to normal sperm counts and increased diploid sperm or oligozoospermia if the anaphase I checkpoint eliminates the abnormal cells. The two 47,XYY patients with abnormal FISH sperm results had severe male factor infertility—one with severe oligoteratozoospermia and the other with azoospermia. Of the three remaining 47,XYY patients with normal FISH sperm results, two were normozoospermic and one was oligozoospermic (mosaic 47,XYY/46,XY karyotype). The normal incidence of aneuploid sperm in the 47,XYY/46,XY patient could be explained by an arrest of the XYY cell line and the normal progression through meiosis of the XY cell line (10).

Although most embryonic abnormalities end in implantation failures or spontaneous abortions, a variable percentage of abnormal offspring has been reported and associated to the presence of aneuploid spermatozoa in the father. In fathers of children with Down's syndrome with a paternal extra chromosome 21, FISH sperm studies have shown elevated incidences of spermatozoa with disomy 21 ranging between 0.75% and 0.78% (42). Similar studies of sperm samples in couples with fetal abortions or children with sex chromosomal abnormalities (Turner syndrome or Klinefelter syndrome) have described increased frequencies of sex chromosome aneuploidies in sperm (between 0.20% and 24.70%) (43–48). We observed 0.59%–1.83% of spermatozoa with sex chromosome disomy in 46,XY patients with this isolated abnormality, whereas

in the two 47,XYY patients with abnormal FISH sperm results, these percentages were 0.56% and 0.77%. These percentages of sperm abnormalities, despite being significantly higher, could be considered relatively low, but the fact is that the incidence of preimplantation embryos with potentially viable sex chromosome abnormalities was threefold and sixfold higher than in fertile population (6.2% and 12.5% vs. 2.0%, respectively). To understand the clinical impact of sperm aneuploidy rates in OAT and azoospermic patients as described in our study and many others, we should not forget that only a selected panel of chromosomes were evaluated. Total aneuploidy rates in OAT patients considering the 24 chromosomes have been estimated to be as high as 33%–74% compared with 4.1%–7.7% in proven fertile donors and total diploidy rates were 0.4%–9.6% in OAT compared with 0.04% in fertile donors (3).

Different hypothesis have been proposed related to the capability of abnormal sperm to fertilize oocytes. Giorlandino et al. (24) reported that nullisomic spermatozoa displayed greater motility than normal sperm and thus, fertilized a higher percentage of oocytes. Although this hypothesis could explain certain types of chromosomopathies, such as Turner syndrome, it could not be applied to trisomy and triploidy. In addition, similar incidences of aneuploid and diploid sperm were described in swim-up motile sperm fractions compared with the pellet fractions in infertile males (49, 50). Other studies focused on sperm morphology described that macrocephalic and multiple tail spermatozoa were mostly abnormal (51–56). However, spermatozoa with normal sperm dimensions and shape can also bear some types of chromosomal abnormalities (57, 58). Therefore, sperm selection based on morphology would allow discarding some, but not all chromosomally abnormal sperm.

Our study has shown elevated rates of chromosomally abnormal embryos in couples with severe male factor infertility (>58 % in all study groups vs. 34% in control group), most of them with complex aneuploidies affecting several chromosomes. Mosaicism was also increased in all study groups ($\geq$30% vs. 7.4% in control group). These results are in agreement with other studies showing high embryo aneuploidy rates ranging from 43%–78% in OAT and azoospermic patients and in patients with abnormal FISH sperm results or meiotic abnormalities (29, 30, 59–62). Mosaicism rate as high as 53% has been reported in patients with nonobstructive azoospermia (59). These findings could be explained by fertilization with sperm carrying multiple chromosomal alterations or centrosome abnormalities. Sperm defective centrosomes impede the formation of asters or lead to the formation of abnormal spindle, with an abnormal distribution of chromosomes, resulting in aneuploid embryos (63). In addition, an abnormal number of male centrioles in the centrosome has been related with the production of haploid, poliploid, or mosaic embryos (59, 64).

In conclusion, we have found a strong correlation between the two types of sperm chromosomal abnormalities and embryo chromosomal constitution in infertile men with 46,XY and 47,XYY karyotypes. Infertile men with increased disomy for sex chromosomes in sperm would have an elevated risk of generating potentially viable aneuploid embryos. Men with increased diploid sperm would have a higher risk of triploid embryos, more related with abortions. Therefore, FISH sperm studies could be a useful tool to provide a more personalized genetic counseling to couples with severe male infertility, with a range of possibilities to offer such as prenatal testing, PGS, or sperm donation.

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