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Semi-Automated Scoring of Triple-probe FISH in Human Sperm: Methods and Further Validation

Melissa J. Perry,[1,2*] Xing Chen,[2] Megan E. McAuliffe,[2] Arnab Maity,[3] Glen M. Deloid[2]

[1]Department of Environmental and Occupational Health, The George Washington University School of Public Health and Health Services, 2100 M Street, NW, Suite 203A, Washington, DC 20037

[2]Department of Environmental Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115

[3]Department of Statistics, North Carolina State University, 2311 Stinson Drive, Raleigh, North Carolina 27695

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*Correspondence to: Melissa J. Perry, ScD, MHS; Department of Environmental and Occupational Health, The George Washington University, School of Public Health and Health Services, 2100 M Street, NW, Suite 203A, Washington, DC 20037

Email: melissa.perry@gwumc.edu

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Abstract

Although the frequency and consequence of sperm chromosomal abnormalities are considerable, few epidemiologic studies in large samples have been conducted to investigate etiologic risk factors. This is, in part, attributable to the labor intensive demands of manual sperm fluorescence *in situ* hybridization (FISH) scoring. As part of an epidemiologic study investigating environmental risk factors for aneuploidy among men attending a hospital-based fertility clinic, a semi-automated method of slide scoring was further validated and used to estimate sex chromosome sperm disomy frequency in a large number of samples. Multiprobe FISH for chromosomes X, Y, and 18 was used to determine sex chromosome disomy in sperm nuclei. Semi-automated scoring methods were used to quantify X disomy (sperm FISH genotype XX18), Y disomy (YY18), and XY disomy (XY18). The semi-automated results were compared with the results from manual scoring in 10 slides. The semi-automated method was then used to estimate sex chromosome disomy frequency in 60 men. Of 10 slides scored, significant differences between the manual and semi-automated results were seen primarily in one slide that was of poor quality because of over swollen nuclei. Among 60 men analyzed using the semi-automated method, median total sex chromosome disomy frequency was 1.65%, which is higher than seen among normal men but within range with reports from fertility clinic populations. These results further validate that semi-automated methods can be used to score sperm disomy with results comparable to manual methods. This is the largest study to date to provide estimates of sex chromosome disomy among men attending fertility clinics. These methods should be replicated in larger clinic populations to arrive at stable estimates of aneuploidy frequency in men who are members of subfertile couples.

Key terms

aneuploidy; chromosomal aberrations; *in situ* hybridization, fluorescence; reproduction; sperm

ALTHOUGH aneuploidy is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies (1), the causes of aneuploidy remain largely unknown. What is known is that autosomal aneuploidies are predominantly maternal (e.g., trisomy 21, 18, 13); while about 55% of aneuploidies of the X or Y chromosomes, which result in Klinefelter (47, XXY) and Turner (45, X) Syndromes, as well as Triple X and XYY aneuploidies come from the father's sperm (1–4). The most common aneuploidies in humans at birth involve abnormal numbers of sex chromosomes (5,6). Children with sex chromosomal abnormalities such as Klinefelter or Turner syndromes may have reproductive disorders, behavioral difficulties, and/or reduced intellectual capabilities compared with their siblings (7).

The influence of environmental factors on the origin of aneuploidy has not been well investigated in epidemiologic studies (8). This can, in part, be attributable to how labor intensive it is to score sperm aneuploidy, which can range from 5–20 h to manually score 10,000 nuclei depending on the experience level of the scorer and the density of spermatozoa on the slides (9). Recent reports have suggested that semi-

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automated methods utilizing computer scoring can be efficiently used to process large numbers of samples with results comparable with manual scoring methods (10–12).

We report here the methods and results of a study further validating semi-automated methods to estimate the occurrence of sperm sex chromosome disomy, as part of a larger study to investigate environmental exposures and sperm aneuploidy among men attending a hospital-based fertility clinic.

Materials and Methods

All study protocols used were approved by the Institutional Review Boards at the Harvard School of Public Health and the Massachusetts General Hospital, and all participants provided written informed consent. Men who were attending the Massachusetts General Hospital (MGH) Andrology Laboratory for fertility evaluation as part of an infertile couple and who were between the ages of 18 and 55 were asked to participate. In approximately 40% of these couples, the fertility problem is associated with the male. Men who had had a vasectomy were not eligible. Because the patient was already providing a semen sample for clinical evaluation, we asked the patient whether the results of the semen sample analysis could be used for the research study. At entry, each participant was asked to complete a self-administered medical history and health behaviors questionnaire on race/ethnicity, medical, and fertility history, and smoking history. All patients were instructed by the clinic (routine pre-visit instructions) to refrain from sexual activity for at least 3 days before providing the semen sample at the clinic, and information on the period of abstinence (in days) was collected at the time of semen collection.

Semen Analysis

Semen was collected and analyzed at MGH in a standardized manner. The semen sample was collected by masturbation in a sterile container and allowed to liquefy for 20 min. The physical properties of the semen, including the sample volume, pH, color, and viscosity were recorded. Sperm counts and percent motility were first determined manually (13), then they were measured by computer-aided semen analysis (CASA) using the Hamilton Thorne Motility Analyzer (IVOS 10). To minimize the variability, we used a constant analysis set-up and performed additional quality control steps including play-back and viewing QC plots in subjects with counts below 20 million/mL and above 50 million/mL (14). To assess sperm morphology (Kruger Strict criteria), seminal smears were made on a glass slide with <5 μL of semen and stained with Diff-Quik.

Sperm Multiprobe Fluorescence *in situ* Hybridization

We used blinded samples to evaluate sex chromosome aneuploidy in sperm nuclei. Sixty samples were available for analysis. A random numbers generator was used to select 10 from the 60 to perform both manual and semi-automated scoring. Chromosome X, Y, and 18 centromere-specific probes were used to assess X, Y, and XY disomy. Chromosome 18 was used as an autosome control. Hybridization was performed using a combination of probes (Vysis, Downers Grove, IL). Chromosome X was hybridized with CEP X (Xp11.1–q11,α satellite) SpectrumGreen, chromosome Y with CEP Y (Yq12 satellite III) SpectrumOrange, and chromosome 18 with CEP 18 (18p11.1–q11.1, α satellite) SpectrumAqua.

Hundred microliter semen samples, in straws, were thawed on ice and diluted in 400 μL PBS + 0.3%BSA, for a concentration of 1:5. After three wash cycles of centrifugation and resuspension, the sperm cell concentration was determined using a hemocytometer and light microscope. Adjustments in concentration were made so as to achieve a final concentration with at least 3×10^5 sperm cells, which allows for the greatest ability to successfully score at least 5,000 nuclei. Ten microliters of sample were cytospun for 1 min at 125rpm onto glass slides. Two slides were prepared for each sample. Slides were checked with a light microscope to ensure that spots had sufficient cells. All samples were allowed to dry and placed in a -20°C freezer until the hybridization process was started.

Hybridization. The hybridization protocol used in this study has been reported previously (11). Briefly, cells were first swollen and then denatured. SpectrumOrange (Y), SpectrumAqua (18), and SpectrumGreen (X) probes were mixed according to Vysis protocol. Ten microliters of the probe mixture were applied immediately to the dense area of cells on the slide and this area was then fitted with a coverslip and sealed with rubber cement. The cells were allowed to hybridize overnight; the next day, the coverslips were removed and the slides were subjected to three washes. 4′,6-diamidino-2-phenylindole (DAPI) II counterstain was applied to the target area of the slide. A coverslip was placed over the area and sealed with clear nail polish. The slides were placed in the freezer at -20°C for at least 30 minutes before viewing under the microscope.

Slide scoring. The validation experiment was designed to capture and score DAPI positive nuclei using the automated system, and then to score the same cells by a trained scorer who was blind to the automated system results. For the semi-automated analysis, we used slide scoring techniques similar to methods reported previously by our group (11) and others (10,12). Slides were imaged by wide field fluorescence microscopy using a BD Pathway 855 Bioimager and Atto Vision imaging software (BD Biosciences, San Jose, CA), with a 40×/0.90 air objective (Olympus, Center Valley, PA). From each slide, 384 non-overlapping image fields were acquired, each consisting of one nuclear (DAPI) and three probe (X, Y, and 18) channel images.

Nuclear (DAPI) images were acquired using 380/20 band-pass (BP) excitation, 400 LP dichroic, 435 long-pass (LP) emission filters. Auto-focusing was performed based on chromosome 18 dye signal, and all images were acquired with flat-field correction and 2×2 binning. Image processing, segmentation, classification, and scoring were performed using custom image processing and analysis software developed in

MATLAB® (The Mathworks, Natick, MA). Classification and scoring algorithms used were designed based on the scoring criteria described by Baumgartner et al. (15).

A Gaussian filter and adaptive histogram equalization were applied to all image channels [DAPI (nuclear), SpectrumAqua (18), SpectrumOrange(Y), and SpectrumGreen(X)] to reduce noise and to correct for nonuniform field brightness. Image intensity threshold values were selected manually to partition images into background and foreground (nuclei or signal) components. Because nuclear and signal intensities varied within images (likely due to variable z position relative to focal plane), automated algorithms for threshold value selection did not produce satisfactory or consistent results, and because background autofluorescence and hybridization signal intensity varied between specimens (likely due to variable physical and biochemical properties), it was not possible to employ a global set of manually selected threshold values across data sets. Threshold values were therefore adjusted visually for each image set (i.e., each specimen) to afford optimal segmentation of nuclei and signals.

Gradient-facilitated watershed segmentation was used to identify and label individual nuclei. Nuclei were excluded if they abutted an image border (the completeness of such nuclei were indeterminate). Nuclei were also excluded if they did not meet the size criterion of having pixel areas falling between 1.5 and 3.0 times the mean area of control unhybridized (unswollen) nuclei. In addition, nuclei were excluded if they failed to meet a set of shape criteria. These criteria were determined empirically in preliminary trials to distinguish single, intact nuclei with normal ellipsoidal morphologies, from clumped, fragmented, or misshapen nuclei. Specifically, nuclei were excluded if they had a roundness value (ratio of perimeter to radius of a circle of equal area, divided by 2π) of less than 0.89, a ratio of major to minor axis length greater than 2.2, or an eccentricity (ratio of inter-foci distance to major axis length) of greater than 0.68.

SpectrumAqua (18), SpectrumOrange (Y), and SpectrumGreen (X) signal images were segmented using a connected-pixel object algorithm to identify individual signal objects.

Colocalization analysis was performed to assign signals to nuclei and to identify nuclei exhibiting disomy or other abnormalities. A signal was assigned to a given nucleus if it was completely contained within it. Signals not contained within a nucleus were discarded from analysis. Disomic cells detected by the system were manually inspected. The total number of signals for each chromosome within each nucleus were subsequently tallied, and disomy X (sperm FISH genotype XX18), disomy Y (YY18), and XY (XY18) were identified. It took approximately 3 h per slide to conduct image analysis and computer scoring. Chromosome 18 was used as an autosome control.

Data Analysis

Patterns of X, Y, and XY disomy were examined and frequencies from the semi-automated system and manual system from the same cells were compared. Because the coordinates for scanned cell positions are recorded by the semi-automated system, the same cells can be relocated easily and scored manually. A slide grid was used to locate the same separate locations on the slide, from which cells were each captured and scored. Ten separate slides (one sample per slide), each with at least 98% hybridization efficiency, were compared and a total of 5,504 cells were scored using both systems. Approximately 2% of cells on each slide were discarded due to a lack of signals. To compare event frequencies using each method, statistical testing was performed using two sample tests of frequency difference with Pearson's chi-square, or Fisher's exact tests when no more than five cells were encountered in a category. We calculated *P* values for comparing the difference between manual and automated methods within disomy conditions on each slide. For estimating disomy among 60 men using semi-automated methods, descriptive statistics for demographic characteristics and semen parameters were calculated. WHO cutoffs were used to categorize concentration ($\geq$20 million/mL), motility ($\geq$50% motile), and morphology ($\geq$4% normal) (14). Median, 25th and 75th percentiles for XX, YY, XY, and total disomy were also calculated.

Results

Table 1 shows comparisons between manual and scoring methods for the frequency of X18, Y18, XX18, YY18, XY18, and total disomy for 10 slides. The number of nuclei scored per slide ranged from 330 to 1,024, and the total number of cells scored by both methods across the 10 slides was 5,504. Significant differences between the manual and semi-automated method were seen primarily in one slide that was of poor quality because of over swollen nuclei. Two other instances were seen in slides 6 and 10 in which differences between total disomy estimates were statistically significant.

Table 2 shows demographic and sperm parameter characteristics of the 60 participants. Average age was 35.6 years, and average days of abstinence before providing a semen sample was 3.9. Percent with below-normal concentration, according to the WHO cut-off of 20 million per mL semen, was 6.7%.

For the analyses using the semi-automated system to calculate disomy estimates for 60 men, a median of 4,350 sperm nuclei were scored per person (range 25th percentile 2,870–75th percentile 6,597) and median total disomy was 1.65%, X disomy was 0.4, Y disomy was 0.33, and XY was 0.83 (Table 3).

Discussion

The results of this study further validate the comparability of semi-automated to manual methods for scoring sperm chromosome aneuploidy. This is the largest study to date to evaluate the semi-automated system on a large sample of men from a fertility clinic.

The main significant discrepancies seen between the manual and automated scoring results were in one slide with over swollen cells. This may be interpreted based on the structure of sperm chromatin. The fundamental packaging unit of sperm chromatin is a toroid, and each toroid contains 60 kilobases of DNA linked to other toroids by uncoiled DNA stretches (16). Overly decondensed sperm may result in the

Table 1. Normal and X and Y disomy frequencies in sperm nuclei using manual versus semi-automated scoring methods of triple-probe FISH

			X18			Y18			XX18			YY18			XY18			TOTAL DISOMY		
SLIDE	METH	*N*	*N*	%	*P*	*N*	%	*P*	*N*	%	*P*	*N*	%	*P*	*N*	%	*P*	*N*	%	*P*
1	Manual	643	290	45.10	0.86	133	20.68	0.83	1	0.16	1	1	0.16	0.62	1	0.46	1	3	0.47	0.34
	Auto	443	294	45.72		137	21.31		2	0.31		3	0.47		2	0.31		7	1.09	
2	Manual	499	196	39.28	0.90	160	32.06	0.68	1	0.20	1	2	0.40	0.68	0	0.00	1	3	0.60	0.73
	Auto	499	199	39.88		153	30.66		1	0.20		4	0.80		0	0.00		5	1.00	
3	Manual	427	202	47.31	1.00	174	40.75	1.00	1	0.23	1	1	0.23	1	0	0.00	1	2	0.47	1
	Auto	427	202	47.31		173	40.52		0	0.00		1	0.23		0	0.00		1	0.23	
4	Manual	411	179	43.55	1.00	161	39.17	0.94	3	0.73	0.62	1	0.24	1	1	0.24	1	5	1.22	1
	Auto	411	179	43.55		159	38.69		1	0.24		2	0.49		1	0.24		4	0.97	
5	Manual	532	178	33.46	0.80	159	29.89	1.00	0	0.00	0.25	4	0.75	0.75	3	0.56	1	7	1.32	0.35
	Auto	532	183	34.40		160	30.08		3	0.56		6	1.13		3	0.56		12	2.26	
6	Manual	517	138	26.69	0.57	129	24.95	0.42	2	0.00	0.50	0	0.00	0.12	4	0.77	0.17	4	0.77	0.01
	Auto	517	129	24.95		117	22.63		0	0.39		4	0.77		10	1.93		16	3.09	
7	Manual	1024	352	34.38	1.00	340	33.20	0.67	0	0.00	1	1	0.10	1	13	1.27	0.58	14	1.37	0.48
	Auto	1024	352	34.38		330	32.23		0	0.00		2	0.20		17	1.66		19	1.86	
8	Manual	620	263	42.42	1.00	276	44.52	0.68	0	0.00	1	0	0.00	0.06	4	0.65	1	4	0.65	0.26
	Auto	620	263	42.42		268	43.23		0	0.00		5	0.81		1	0.65		9	1.45	
9	Manual	330	101	30.61	0.001	99	30.00	0.04	3	0.91	0.09	1	0.30	0.12	0	0.00	0.50	4	1.21	0.01
	Auto	330	58	17.58		75	22.73		10	3.03		6	1.82		2	0.61		18	5.45	
10	Manual	501	231	46.11	0.89	164	32.73	0.63	0	0.00	0.25	0	0.00	0.50	2	0.40	0.28	2	0.40	0.02
	Auto	501	228	45.51		172	34.33		3	0.60		2	0.40		6	1.20		11	2.20	

Meth = automated or manual scoring; *P* = Value for comparison of each slide using Pearson's chi-square or Fisher's exact tests.

Table 2. Age and sperm parameters[a] of study participants (n = 60)

DESCRIPTIVE CHARACTERISTIC	MEAN OR %
Mean age in years	35.6
(SD) (range)	(5.43) (24–50)
Mean abstinence time in days	3.9
(SD) (range)	(2.35) (1–14)
% Concentration (n)	
<20 million/mL	6.7 (4)
>20 million/mL	93.3 (56)
Motility (n)	
<50% motile	48.3 (29)
>50% motile	51.7 (31)
Morphology (n)	
<4% normal	11.7 (7)
≥4% normal	88.3 (53)

[a] Based on World Health Organization semen analysis cutoffs (14).

uncoiling of DNA into toroidal subunits which can give the faulty appearance of multiple centromeric signals, when they are in fact DNA pieces from the same centromere in the toroid that has unraveled during decondensation, resulting in inflated disomy and diploidy estimates. Under these circumstances, estimates are likely to be inaccurate regardless of scoring method; and in this particular instance the semi-automated system produced higher disomy estimates than manual methods. Slides 6 and 10 also showed significant differences between manual and automated methods in total disomy estimates only, but not in individual disomy conditions. This may be the result of small nonsignificant differences in the three different disomic conditions accumulating in significant differences in total disomy in these two slides.

Prior reviews of sperm fluorescence *in situ* hybridization (FISH) results in normozoospermic men have reported the following ranges (%): X disomy: 0.03–0.37; Y disomy: 0.04–0.21; XY: 0.06–0.42 (17). Another review reported that percent total disomy for the sex chromosomes averaged across 23 studies of normozoospermic men using multicolor FISH was 0.26 (18). The disomy frequency estimates among the 60 men in this study were higher than these previously reported ranges. However, considerable differences in disomy frequency for specific chromosomes have been identified (19) and are potentially attributed to inter-donor heterogeneity and/or different methodologies used among laboratories (primarily the type of DNA probes used, the scoring criteria applied, or the number of sperm analyzed) (18,20).

Because the previously reported ranges are from studies on normal men using manual cell counting methods, whether the higher disomy seen in this study is attributable to actual higher disomy prevalence in a clinic sample or differences in our semi-automated methods for estimating percent disomy is unclear. For example, in a study of fertility clinic patients who were known reciprocal translocation carriers, XY disomy was as high as 4.1% in one patient (21). Although the majority of the men in our sample were normal, some men did have abnormal semen parameters and it is known that chromosome anomalies are more frequent in infertile males than in the general population (22). Analyses of disomy by semen parameters from this study are underway, with preliminary results suggesting an inverse association between sperm concentration and disomy but without clear associations with motility and morphology (unpublished data). Prior studies have found inverse associations between disomy and all three of these parameters separately (23–25). Because karyotypes were not routinely performed on the men in our study, we do not know the extent to which they had underlying genetic conditions such as translocations that could increase overall disomy prevalence.

This work builds on our previous validation work comparing automated with manual results. We previously compared similar methods for scoring sex chromosome disomy in a sample of four normozoospermic men from a fertility clinic (11). In that study, we also found higher disomy estimates than previously reported, and as seen here, results did not differ between manual and semi-automated methods. A recently reported study of sperm disomy among andrology clinic patients using automated methods also did not find differences between manual and automated estimates and also reported higher sex chromosome disomy averaging between 1.3 and 1.5% total disomy (12). However, an andrology clinic study by Carrell and Emery (10) using automated methods reported the mean aneuploidy rate to be 1.59% + 0.21% (standard error of the mean) for chromosomes 13, 18, 21, X, and Y combined, comparable with previously reported ranges from normozoospermic men using manual scoring methods. Although this validation experiment improves upon our previous work by studying more participants, disomy frequencies from this experiment should still be interpreted cautiously because a small number of total nuclei were scored per participant (median = 525), and estimates of rare events become more reliable as the number of observations increase.

Continued work in optimizing, validating, and replicating automated methods is needed (26) particularly for the accurate determination of diploidy (12,27). However, the use of a validated semi-automated method for estimating disomic sperm frequencies was considered a strength in this study because it allowed for objective processing of a large number of samples. At least one study has provided qualitative evidence that automated methods may be more accurate than manual methods, particularly in the case of diploid cells where

Table 3. Sperm nuclei and percent disomy scored among total sample (N = 60)

SPERM OUTCOMES	MEDIAN	25TH PERCENTILE	75TH PERCENTILE
Nuclei (n)	4350	2870	6597
Total disomy	1.65	1.10	2.77
XX18	0.40	0.19	0.74
YY18	0.33	0.21	0.57
XY18	0.83	0.46	1.63

digital capturing and thresholding can reduce the erroneous appearance of two signals caused when two separate cells are pressed together (12). Also, at least one report suggests that flow cytometry methods can be used to identify aneuploid sperm (28), which could also potentially offer high-throughput advantages. It is important that these high-throughput semi-automated methods be replicated in additional studies and in large sample sizes to arrive at stable estimates of aneuploidy frequency among men who are members of subfertile couples.

Acknowledgments

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Literature Cited

1. Hassold T, Hunt P. To err (meiotically) is human: The genesis of human aneuploidy. Nat Rev Genet 2001;2:280–291.
2. Chandley AC. On the parental origin of de novo mutation in man. J Med Genet 1991;28:217–223.
3. Hall H, Hunt P, Hassold T. Meiosis and sex chromosome aneuploidy: How meiotic errors cause aneuploidy; how aneuploidy causes meiotic errors. Curr Opin Genet Dev 2006;16:323–329.
4. Marchetti F, Wyrobek AJ. Mechanisms and consequences of paternally-transmitted chromosomal abnormalities. Birth Defects Res C Embryo Today 2005;75:112–129.
5. Martin RH, Ko E, Rademaker A. Distribution of aneuploidy in human gametes: Comparison between human sperm and oocytes. Am J Med Genet 1991;39:321–331.
6. Shi Q, Martin RH. Aneuploidy in human sperm: A review of the frequency and distribution of aneuploidy, effects of donor age and lifestyle factors. Cytogenet Cell Genet 2000;90:219–226.
7. Martin RH. Meiotic chromosome abnormalities in human spermatogenesis. Reprod Toxicol 2006;22:142–147.
8. Wyrobek AJ, Schmid TE, Marchetti F. Cross-species sperm-FISH assays for chemical testing and assessing paternal risk for chromosomally abnormal pregnancies. Environ Mol Mutagen 2005;45:271–283.
9. Martin RH, Greene C, Rademaker AW, Ko E, Chernos J. Analysis of anueploidy in spermatozoa from testicular biopsies from men with nonobstructive azoospermia. J Androl 2003;24:100–103.
10. Carrell DT, Emery BR. Use of automated imaging and analysis technology for the detection of aneuploidy in human sperm. Fertil Steril 2008;90:434–437.
11. Perry MJ, Chen X, Lu X. Automated scoring of multiprobe FISH in human spermatozoa. Cytometry A 2007;71A:968–972.
12. Tempest HG, Cheng SY, Gillott DJ, Handyside AH, Thornhill AR, Griffin DK. Scoring of sperm chromosomal abnormalities by manual and automated approaches: Qualitative and quantitative comparisons. Asian J Androl 2009;12:257–262.
13. Davis RO, Katz DF. Review: Operational standards for CASA instruments. J Androl 1993;14:385–394.
14. World Health Organization (WHO). WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th ed. New York: Cambridge University Press; 1999.
15. Baumgartner A, Van Hummelen P, Lowe XR, Adler I-D, Wyrobek AJ. Numerical and structural chromosomal abnormalities detected in human sperm with a combination of multicolor FISH assays. Environ Mol Mutagen 1999;33:49–58.
16. Fuentes-Mascorro G, Serrano H, Rosado A. Sperm chromatin. Arch Androl 2000;45:215–225.
17. Egozcue J, Blanco J, Vidal F. Chromosome studies in human sperm nuclei using fluorescence in-situ hybridization (FISH). Hum Reprod Update 1997;3:441–452.
18. Templado C, Bosch M, Benet J. Frequency and distribution of chromosome abnormalities in human spermatozoa. Cytogenet Genome Res 2005;111:199–205.
19. Moore DH II, Epstein L, Reeder J, Wheeless L, Waldman FM. Interlaboratory variability in fluorescence in situ hybridization analysis. Cytometry 1996;25:125–132.
20. Schmid TE, Lowe X, Marchetti F, Bishop J, Haseman J, Wyrobek AJ. Evaluation of inter-scorer and inter-laboratory reliability of the mouse epididymal sperm aneuploidy (m-ESA) assay. Mutagenesis 2001;16:189–195.
21. Moretti E, Pascarelli NA, Giannerini V, Geminiani M, Anichini C, Collodel G. 18,X,Y aneuploidies and transmission electron microscopy studies in spermatozoa from five carriers of different reciprocal translocations. Asian J Androl 2009;11:325–332.
22. Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A, Aran B, Barri PN, Vidal F, Egozcue J. Human male infertility: Chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. Hum Reprod Update 2000;6:93–105.
23. Collodel G, Capitani S, Baccetti B, Pammolli A, Moretti E. Sperm aneuploidies and low progressive motility. Hum Reprod 2007;22:1893–1898.
24. Machev N, Gosset P, Viville S. Chromosome abnormalities in sperm from infertile men with normal somatic karyotypes: Teratozoospermia. Cytogenet Genome Res 2005;111:352–357.
25. Martin RH, Rademaker AW, Greene C, Ko E, Hoang T, Barclay L, Chernos J. A comparison of the frequency of sperm chromosome abnormalities in men with mild, moderate, and severe oligozoospermia. Biol Reprod 2003;69:535–539.
26. Levsky JM, Singer RH. Fluorescence in situ hybridization: Past, present and future. J Cell Sci 2003;116(Pt 14):2833–2838.
27. Molina O, Sarrante Z, Vidal F, Blanco J. FISH on sperm: Spot-counting to stop counting? Not yet. Fertil Steril 2009;92:1474–1480.
28. Kovacs T, Bekesi G, Fabian A, Rakosy Z, Horvath G, Matyus L, Balazs M, Jenei A. DNA flow cytometry of human spermatozoa: Consistent stoichiometric staining sperm DNA using a novel decondensation protocol. Cytometry A 2008;73A:965–970.