

Analysis of chromosome aneuploidy in sperm by fluorescence in situ hybridization — a new approach to the study of male fertility in environmental exposures

by Jaana Lähdetie, MD,^{1,2} Solveig B Larsen, MD,³ Kati Härkönen, MD,¹ Asclepios⁴

Lähdetie J, Larsen SB, Härkönen K, Asclepios. Analysis of chromosome aneuploidy in sperm by fluorescence in situ hybridization — a new approach to the study of male fertility in environmental exposures. *Scand J Work Environ Health* 1999;25 suppl 1:26—27.

Key terms aneuploidy, chromosomes, fungicides, sperm.

In humans, aneuploidy (ie, abnormality of the chromosome number) causes serious reproductive problems, for example, spontaneous abortions and congenital malformations in newborns. A lack of useful methods has hampered the study of genotoxic effects on human gametes. During the last decade, however, fluorescence in situ hybridization (FISH) with chromosome-specific probes has become a useful tool to study the chromosome number of human spermatozoa because it allows a large number of cells to be analyzed.

The aim of this study was to analyze the chromosome number of sperm in semen samples of farmers exposed to fungicides. Fungicides, especially some carbamates, have been shown to produce aneuploidy in somatic cells (1).

The samples were collected at a mobile laboratory in Denmark in February-May (preexposure) and in August-September (postexposure). The median time from the

start of fungicide exposure to the second sample was 92 (range 56—128) days. The farmers answered a detailed questionnaire on both occasions by responding to questions related to their exposure to fungicides and to their personal habits (eg, smoking and alcohol consumption). A standard semen analysis was performed in the laboratory. A semen sample (300 µl) was frozen at -70°C and stored for the FISH analyses. Semen samples of the 34 most heavily exposed farmers were sent on dry ice to the laboratory in Finland. The samples were prepared as described earlier (2).

Chromosome-specific probes for chromosomes 1 and 7 were used because they could be made in the laboratory and thus were economic for large-scale use. The chromosome 1 signal was detected with red fluorescence and the chromosome 7 signal with green, while the background nuclear staining was weak red. The slides were coded and examined in a fluorescence microscope

1 Department of Medical Genetics, University of Turku, Turku, Finland.

2 Department of Child Neurology, University of Turku, Turku, Finland.

3 Department of Occupational Medicine, University of Aarhus, Aarhus, Denmark.

4 The Asclepios project on occupational hazards to male reproductive capability is a biomedical research project of the European Union that was carried out in 14 European centers in 1993—1998. The project was coordinated by The Steno Institute of Public Health, University of Aarhus Denmark, and it included the following researchers: Belgium, Gent (P Kiss, A Mahmoud, M Vanhoorne, H Verstraelen); Denmark, Aarhus (A Abell, JP Bonde, SB Larsen, G Danscher, E Ernst, H Kolstad), Copenhagen (A Giwercman); England, London (A Dale, M Joffe, N Shah); Finland, Helsinki (M-L Lindbohm, H Taskinen, M Sallmen), Turku (J Lähdetie); France, Paris (P Jouannet, P Thonneau), Strasbourg (A Clavert); Germany, Erlangen (KH Schaller, W Zschiesche); Italy, Brescia (P Apostoli, S Porru), Milano (L Bisanti), Pietrasanta (L Lastrucci), Rome (M Spanò); The Netherlands, Nijmegen (N Roeleveld, H Thuis, GA Zielhuis), Zeist (W de Kort); Poland, Lodz (K Sitarek).

equipped with a double-band-pass filter that allowed the simultaneous detection of both red and green fluorescence.

Altogether 10 000 spermatozoa were scored by 2 trained scorers, each scoring 5000 spermatozoa. The frequency of spermatozoa with 0, 1, or 2 red and 0, 1, or 2 green signals was determined.

The scoring criteria were as described earlier (2). A normal haploid sperm cell shows 1 red and 1 green signal. Hyperhaploid sperm nuclei contain more than 1 spot of either red (disomy-1) or green (disomy-7) fluorescence. Diploid sperm show 2 signals of both colors. The sum of the hyperploid sperm (disomy-1 + disomy-7 + diploidy) was also determined. When the hybridization efficiencies were calculated, the sperm with no signals or only 1 signal of either color were considered indicators of a lack of hybridization although some of these cells may have represented true nullisomy.

The statistical analysis included a comparison of the sum of hyperploid sperm before and after exposure with the use of a paired t-test (the sum was normally distributed), whereas the disomy and diploidy rates were not normally distributed and were compared with nonparametric methods using SAS statistical program package 8 (3).

The analysis of the semen samples of the farmers occupationally exposed to fungicides was successful for 65 of the 68 (95.6%) samples obtained. The hybridization efficiency was excellent (98.7%). The analysis of the effect of life-style factors on the sperm chromosome aneuploidy by FISH will be reported separately.

Fungicide exposure did not significantly alter the frequencies of aneuploid sperm. The frequency of sperm disomic for chromosome 1 was 11.9×10^{-4} before and 11.8×10^{-4} after the exposure, for disomy-7 the corresponding values were 4.9×10^{-4} and 4.5×10^{-4} , for diploidy the values were 10.2×10^{-4} and 9.0×10^{-4} , and for the sum of the hyperploid sperm the corresponding values were 27.0×10^{-4} and 25.3×10^{-4} . The frequencies are in accordance or slightly lower than previous results ob-

tained from fertile men studied in our laboratory and showing disomy-1 in 11.5×10^{-4} , disomy-7 in 6.4×10^{-4} , and diploidy in 15.0×10^{-4} spermatozoa (2).

FISH methods have obtained increasing popularity in genetic toxicology. It is still too early to draw conclusions on the usefulness of the analysis in occupational studies on male fertility. The experience gained from our study shows that FISH is a rather laborious and expensive technique and, at present, is not easily applicable to large, population-based studies. Automated, image-analysis-based scoring methods would be very welcome.

Human exposures that have been shown to induce an increase in sperm aneuploidy include cytostatic drugs (4) and alcohol and caffeine consumption (5). Fungicide exposure did not alter the sperm aneuploidy frequencies although this group of chemicals contains some known aneugenic agents. Further studies are needed to determine whether occupational exposures can induce aneuploidy in human sperm.

Acknowledgments

Mari Ajosenpää-Saari and Timo Saarinen are acknowledged for their skillful technical assistance and Tommi Viitanen for his statistical analysis of the data.

References

1. Barale R, Scapoli C, Meli C, Casini D, Minunni M, Marrazzini A, et al. Cytogenetic effects of benzimidazoles in mouse bone marrow. *Mutat Res* 1993;300:15–28.
2. Lähdetie J, Ajosenpää-Saari M, Mykkänen J. Detection of aneuploidy in human spermatozoa of normal semen donors by fluorescence in situ hybridization. *Environ Health Perspect* 1996;104 suppl 3:629–32.
3. SAS Institute Inc. SAS/STAT user's guide, version 6. Cary (NC): SAS Institute, 1990.
4. Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier HU, Cassel MJ, et al. Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. *Nature Genet* 1997;16:74–8.
5. Robbins WA, Vine MF, Truong KY, Everson R. Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. *Environ Mol Mutagen* 1997;30:175–83.