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Advances in understanding paternally transmitted chromosomal abnormalities

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Abstract

Multicolor FISH has been adapted for detecting the major types of chromosomal abnormalities in human sperm including aneuploidies for clinically-relevant chromosomes, chromosomal aberrations including breaks and rearrangements, and other numerical abnormalities. The various sperm FISH assays have been used to evaluate healthy men, men of advanced age, and men who have received mutagenic cancer therapy. The mouse has also been used as a model to investigate the mechanism of paternally transmitted genetic damage. Sperm FISH for the mouse has been used to detect chromosomally abnormal mouse sperm, while the PAINT/DAPI analysis of mouse zygotes has been used to evaluate the types of chromosomal defects that can be paternally transmitted to the embryo and their effects on embryonic development.

1. The paternal contribution to abnormal reproductive outcomes

Chromosomal abnormalities transmitted through gametes are associated with pregnancy loss, infant mortality, developmental and morphological defects, infertility and genetic diseases including cancer (1). The social and medical costs of reproductive abnormalities are formidable, yet their causes are not well understood (2). However, it is known that numerical and structural chromosome aberrations are an important contributor (3, 4). Annually in the United States, nearly 2 million conceptions (assuming 4.2 million births per year) are lost before the 20th week of gestation, and of these ~50% carry chromosomal abnormalities (5). The frequency of chromosomal abnormalities among newborns in the U.S. is ~0.6%, including aneuploidies and structural abnormalities.

Chromosomal abnormalities in offspring are typically de novo events that originate in the germ cells of one of the parents or early after fertilization, when they are likely to result in mosaicism. Cytogenetic markers and molecular techniques have made it possible to determine the parent of origin of the abnormal chromosome(s) in affected offspring and are beginning to help us associate the parental origin of specific abnormalities with embryonic fates. The relative parental contribution to chromosomal abnormalities in offspring seems to vary by the defect involved. While constitutive trisomies (e.g., trisomy 21, 18, 13) have large maternal contribution (6), the male contribution is more substantial for sex chromosomal aneuploidies (7, 8). In addition, over 80% of de novo structural chromosomal abnormalities among liveborn appear to be paternally derived (3, 9). Despite the health risk to the developing embryo and offspring,

little is known about the etiology of paternally-derived structural chromosomal abnormalities.

The human-sperm/hamster-egg cytogenetic method (or hamster-egg method) has become the reference method for examining the chromosomal constitution of human sperm. It is based on the fertilization of capacitated human sperm with hamster oocytes whose zona pellucidae were removed enzymatically. Originally described by Rudak et al. (10), this method allows human sperm chromosomes to be examined at the first metaphase after fertilization in the hamster zygote using standard cytogenetic staining techniques (11-19). Using the hamster-egg system, significant variation was found in the frequencies of chromosomally abnormal sperm among healthy men, and these inter-donor differences were reproducible over time. Moreover, men exposed to certain genotoxic agents (i.e., chemotherapy and radiation) exhibited higher frequencies of sperm with chromosomal aberrations compared to controls (20-23). Consistent findings among laboratories for healthy men have been unexpectedly high frequencies of sperm with structural chromosomal abnormalities (5%-13%) compared to aneuploidies (1-3%) and the presence of more chromosomal breaks and fragments than rearrangements. Brandriff et al. (13) and Estop et al. (17) suggested that these breaks originated somewhere between the end of male meiosis and the beginning of the S-phase of the first cell cycle after fertilization but could not determine whether they pre-existed in the fertilizing sperm, owing to an inherent limitation of that methodology. Moreover, the hamster-egg method has been difficult and expensive to perform.

2. Advances in the detection of chromosomally abnormal sperm

During the past decade, fluorescence *in situ* hybridization (FISH) has evolved into an efficient molecular cytogenetic approach for detecting sperm aneuploidy in healthy and exposed men (4, 5, 24) and has facilitated the analysis of much larger numbers of sperm than was previously possible with the hamster-egg method. Since its introduction, sperm FISH technology has made several gradual advances. Research emphasis has shifted from using any chromosome for which a DNA probe is available to chromosomes with clinical relevance in human aneuploidy syndromes (i.e., 21, 18, 13, X and Y) or associated with spontaneous abortions (e.g., 16). A second advance was the adaptation of FISH for the detection of sperm carrying chromosomal rearrangements. Van Hummelen et al. (25) developed a FISH method, using DNA probes to detect human sperm carrying three types of chromosomal defects: (a) terminal duplications and deletions in chromosome 1p (i.e. segmental aneuploidies) which are the sperm products of premeiotic or meiotic chromosomal breakage events or rearrangements; (b) aneuploidy involving two autosomes, and (c) diploidy. The average frequency of sperm with terminal duplications and deletions of 1p by FISH was 6.1 per 10^4, which was significantly higher ($P < 0.01$) than the 2.1 per 10^4 sperm with chromosome 1 aneuploidy (i.e., disomy, nullisomy). A good correlation was obtained between the frequencies of sperm with structural rearrangements by FISH and the hamster-egg method for a reciprocal translocation carrier (26).

The majority of aberrations detected in sperm from healthy men by the hamster-egg method were chromosomal breaks and fragments. The hamster-egg method, however, could not determine whether these breaks occurred before or after fertilization. Sloter et al. (27) recently adapted multicolor FISH by integrating three concepts of prior work into

a single procedure (Table 1, Figure 1A): (a) the detection of 1p duplications and deletions in sperm (25); (b) the measurement of breaks in 1q12, as developed by Eastmond et al. (28) for lymphocytes, and (c) aneuploidy analyses by sperm FISH (4). This new FISH methodology (i.e., sperm ACM method, which utilizes DNA probes specific for the alpha [1cen], classical [1q12], and midisatellite [1p36.3] regions of chromosome 1) also detects duplications and deletions of 1cen-1q12, provides the distribution of breakpoint locations within the ~15Mb 1cen-1q12 region in sperm, and provides data for comparisons of the frequencies of these various chromosomal defects in sperm within a single analysis.

In healthy men, the average frequencies of sperm with partial chromosomal duplications and deletions (e.g., Figure 1E) by sperm ACM were: (a) 4.5 ± 0.5 and 4.1 ± 1.3 per 10^4 involving 1pter, and (b) 0.9 ± 0.4 and 0.8 ± 0.3 per 10^4 involving 1cen, respectively. Duplications to deletions of 1p and duplications to deletions of 1cen each occurred in ~1:1 ratios. This symmetry may be a product of meiotic segregation of reciprocal translocations involving 1p which would be expected to produce equal frequencies of 1p duplications and deletions (i.e., adjacent I segregation), and more rarely, equal frequencies of sperm with duplications and deletions of 1cen (i.e., adjacent II). Under the assumption that all duplications and deletions measured by the sperm ACM assay are derived from reciprocal translocations that are randomly distributed along the genome, we predicted that 0.5-0.6% of sperm may carry a balanced translocation. However, the frequency of reciprocal translocations is estimated to be only ~0.1% among newborns and ~0.2% in prenatal studies (29). The difference between the frequency of reciprocal translocations observed among offspring and the predicted values obtained by sperm-FISH may be due, in part, to *in utero* selection, a non-random distribution of

translocations within the genome, or an overestimation in sperm due to the presence of chromosomal inversions, acentric fragments, or unequal crossing over in spermatocytes. Recently, FISH analysis of seven unrelated patients revealed de novo duplication of 17p11.2 (i.e., the recombination reciprocal product of the Smith-Magenis 17p11.2 deletion syndrome) which arose from unequal crossing over between homologous chromosomes preferentially during spermatogenesis (30). Although unequal crossing over and pericentric inversions involving 1p might produce symmetrical frequencies of duplications and deletions of 1p36.3 in sperm, they are not predicted to produce duplications or deletions of the 1cen-1q12 region. Also, paracentric inversions would not be expected to yield the duplication and deletion phenotypes shown in Table 1. Thus, further studies are warranted to determine whether the ACM assay is a valid measure of rare cells that arise from stem cells or meiotic cells that carry reciprocal translocations. Acentric fragments are an ambiguous category of damage observed using the hamster-egg method that cannot be resolved with confidence using the sperm ACM assay.

Comparison of the ACM and hamster-egg methods was used to validate the ACM method. Sperm carrying breaks within the 1cen-1q12 region (i.e., A-C-M and AC-C-M FISH phenotypes; e.g., Figures 1C-D) comprised 58% of all structural abnormalities detected by the ACM assay. These phenotypes could represent chromosomal breaks, translocations, inversions, or partial duplications involving the 1cen-1q12 region. In the hamster-egg system, chromosomal breaks were the only type of aberration affecting the 1cen-1q12 region and occurred with a frequency of 17.8 per 10^4 sperm metaphases (11, 12, 16, 19, 31). When compared against the ACM assay, this frequency was not

statistically different (P = 0.5) from the 14.1 ± 1.2 breaks per 10^4 sperm detected by FISH.

The strong agreement between the frequency of breaks within the 1cen-1q12 region measured by FISH and the hamster-egg method provides some evidence that chromosomal breaks already existed within the fertilizing sperm, and that few, if any, were induced after fertilization. Furthermore, this interpretation suggests that there may be little selection at fertilization against sperm carrying chromosome breaks, which contradicts findings using certain animal models (32, 33), but supports the findings of Van Hummelen et al. (26) for a reciprocal translocation carrier and Honda et al. (34) for a Robertsonian carrier.

The aggregate frequency of chromosomal breaks, duplications, and deletions detected for chromosome 1 was 24.8 per 10^4 sperm. These structural aberrations accounted for 71% of the abnormalities detected by sperm ACM, which was significantly higher ($P < 2x10^{-8}$) than the 10.1 per 10^4 sperm carrying numerical abnormalities of chromosome 1 (i.e., disomy, nullisomy, and diploidy; e.g., Figure 1A).

3. Applications of sperm FISH to assess paternal risk factors

Using prior FISH methodologies for sperm aneuploidy, several paternal risk factors have been identified for sperm disomies, such as paternal age (35-37), chemotherapy (23, 38, 39), and cigarette smoking (24, 40). Our method for detecting chromosomal breaks and rearrangements in sperm provides an important new approach for measuring exposure to chromosome-breaking agents and assessing genetic predisposition to such damage. Pairing our ACM assay with multicolor FISH assays for sperm aneuploidy

(e.g., the X-Y-21-18 assay (26)) promises to provide a robust approach for detecting paternally transmissible chromosomal damage of both the numerical and structural types.

4. The need for rodent FISH sperm models

While numerous multicolor sperm FISH assays have been developed to detect aneuploidy in humans and rodents, methods for detecting structural aberrations in sperm are available only for humans. Recently, our lab developed a corollary sperm FISH method for the mouse to detect partial chromosomal duplication and deletions incvolving chromosomes 2. Initial findings suggest that the incidence of mouse sperm carrying chromosomal aberrations is 2.5 fold lower than that found in man (41). As FISH assays for detecting structural chromosome aberrations in sperm continue to be developed and applied in various species, it will become increasingly interesting to contrast animal-to-animal variations within species, variations among species, and the relative differences among species in their response to environmental exposures or physiological factors (e.g., age, diet).

5. Mouse methods for studying transmitted chromosomal aberrations

The mouse is an important animal model in pharmacology, reproductive toxicology, and cancer research, and its metabolism and physiology have been well characterized. It is well known that when male mice are treated with a germinal mutagen and mated with unexposed females, the deleterious effects on reproduction can be dramatic, including embryonic dominant lethality, heritable translocations, and malformations, as well as cancer in the offspring. Historically, the identification and characterization of

mammalian germ cell mutagens that may pose a genetic hazard for humans has relied on the use of the dominant lethal (DL) and the heritable translocation (HT) tests in mice. The DL test measures the induction of mutations that lead to embryonic death of the progeny of treated males, while the HT test measures the induction of chromosomal rearrangements in the offspring of treated males (42 and references therein; 43). Additionally, the specific locus (SL) test has been used to generate data on induced gene mutations. As shown in Figure 2, there are 11 chemicals for which DL and HT data are available. With the exception of etoposide, all chemicals produced the highest DL response during the postmeiotic stage of spermatogenesis. The high sensitivity of the postmeiotic stage to chemical exposure is probably related to the reduced DNA repair capacity of sperm and late spermatids as compared with early spermatids and the other spermatogenic cell types (44). Therefore, damage induced in these late stages of spermatogenesis may accumulate and be transmitted. Regarding HT, all chemicals produced a significant increase in the frequencies of reciprocal translocations among the offspring, however there were quantitative differences. While there is a close relationship between the rates at which DL and HT mutations are induced for the majority of the chemicals studied, 3 of the 11 chemicals (etoposide, isopropyl methanesulphonate and methyl methanesulphonate) were strong inducers of DL but only weak inducers of heritable translocations.

The reasons for these differing responses are not fully understood. Our knowledge of the mechanisms that are involved in the conversion of chemically induced DNA lesions in male germ cells into genetic aberrations and their transmission to the offspring is very limited. There is evidence that the nature of the mutations is dependent upon the germ

cell stage rather than upon the chemical administered (45) and that there are differences in the ability of the eggs of different strains to repair the sperm (46). However, because the DL test often cannot to provide information on the mechanism(s) responsible for the observed effects, more direct methods are required to understand the interplay between nature of the mutagen used, the germ cell stage exposed and its repair status and the genetic outcome.

6. Cytogenetic analysis of mouse first-cleavage zygotes

The cytogenetic analysis of mouse first-cleavage (1-Cl) zygotes has long been used for measuring the induction of aneuploidy after treatment of female germ cells (47) and the induction of chromosomal structural aberrations after treatment of male germ cells with alkylating agents (48-51), radiomimetics (52) or irradiation (53). An advantage of the zygote analysis is the possibility of identifying the parental origin of the abnormality because the paternal and maternal chromosomes do not join until the metaphase stage of the first mitotic division (54) and because the maternal chromosomes show a higher degree of condensation with respect to the paternal chromosomes (55). We previously reported the development of a methodology (PAINT/DAPI analysis) for detecting classes of chromosomal aberrations in mouse first-cleavage (1-Cl) zygotes that are predictive of spontaneous abortions and offspring with chromosomal abnormalities (56). This procedure combines DAPI staining, to detect unstable aberrations such as dicentrics and acentric fragments (Figure 1F), with chromosome-specific FISH painting probes to detect stable aberrations such as translocations and insertions (Figure 1G).

7. PAINT/DAPI analysis after paternal exposure to acrylamide

We applied the PAINT/DAPI analysis to study the induction and transmission of stable and unstable chromosomal aberrations in 1-Cl zygotes after treatment of male mice with acrylamide (57). The objectives were to determine whether PAINT/DAPI analysis provided reliable estimates of DL and HT frequencies compared with results obtained using standard breeding methods. Acrylamide (AA) induces chromosomal damage at first-cleavage (51, 56, 57), abnormalities in preimplantation development (58, 59), DL (60), HT (61-63) and specific locus mutations (64, 65), particularly after treatment of late spermatids and early spermatozoa. These cell types are highly sensitive to AA because, in addition to the reduced repair capacity of these late stages, protamines, basic proteins that replace histones during mid-spermiogenesis and are the predominant nuclear proteins during the late postmeiotic stages (66), are considered to be the primary site of AA-alkylation (67).

Male mice treated with 5 consecutive daily doses of 50 mg/kg acrylamide were mated with untreated females at various intervals after the last acrylamide injection to allow comparisons with published DL and HT data. As shown in Figure 3, the number of zygotes with unstable aberrations provided estimates of dead implants that agreed both in magnitude and in kinetics with the results obtained in the DL test. Also, the proportion of zygotes with stable aberrations was comparable with the frequencies of offspring with heritable translocations reported using the standard HT method (28% versus 38%, respectively). These results strongly suggested that chromosomal aberrations are the main cause of embryonic lethality following chemical treatment of male germ cells.

The favorable comparisons of PAINT/DAPI analysis with DL and HT data suggest that the former is a promising method for identifying germ cell mutagens and provides more mechanistic information than the standard DL and HT tests. Potential advantages of PAINT/DAPI analysis of zygotes would be the simultaneous collection of data predictive of DL and HT outcomes, the elucidation of the cytogenetic mechanisms responsible for the observed outcomes, and also a dramatic reduction in the number of animals required for conducting the tests. However, before replacing the current tests, additional experiments in zygotes must be conducted to determine whether the correlation between unstable aberrations and dominant lethality and stable aberrations and heritable translocations holds true for other chemicals, especially for those with unusual patterns of germ cell specificity and differing DL and HT outcomes.

8. Future directions

We have described the advances in FISH for detecting sperm carrying chromosome structural aberrations and aneuploidy in humans as well as rodents. We now have an extensive battery of assays that, at least in humans, offers the possibility of studying aneuploidy induction for clinically-relevant chromosomes (i.e., those that are compatible with postnatal life) and of chromosome structural aberrations induced at any stage of human spermatogenesis (i.e., premeiosis, meiosis and post meiosis. A necessary next step is the development of similar assays in various species including the mouse and rat. The applicability of FISH to many species opens the possibility to investigate relative differences among species in their response to physiological and environmental factors.

In addition to these sperm-based assay, the PAINT/DAPI analysis of mouse zygotes can be applied to understanding the mechanisms responsible for the differing sensitivity of the various germ cell stages to the induction and transmission of chromosomal aberrations to the offspring. An area for improvement is the sensitivity of the assay which at can be achieved by increasing the number of chromosomes that can be painted at a given time thereby augmenting the number of exchanges detected. As shown in Figure 1I, we have recently developed a new probe combination that allows the detection of ~50 of all chromosomal exchanges. Additionally, the increasing number of mice with specific gene mutations related to DNA metabolism and meiosis provides a tool for understanding the mechanisms of male germ cell genotoxicity, germ-cell stage sensitivities, the DNA repair capacity of the fertilized egg, and the risk for paternally transmitted chromosomal abnormalities.

References

1. Wyrobek AJ, Marchetti F, Sloter E, and Bishop J. Chromosomally defective sperm and their developmental consequences. In: Human Monitoring after Envrionmental and Occupational Exposure to Chemical and Physical Agents, Vol. 313, (D. Anderson, A. E. Karakaya, and R. J. Sram, eds.), Amsterdam:IOS press, 2000;134-150.

2. Hook EB. The impact of aneuploidy upon public health: mortality and morbidity associated with human chromosome abnormalities. In: Aneuploidy: Etiology and Mechanisms, (V. Dellarco, P. E. Voytek, and A. Hollaender, eds.), New York:Basic Life Sciences, 1985;7-34.

3. Chandley AC. On the paternal origin of de novo mutation in men. J Med Genet. 28:217-223, 1991.

4. Wyrobek AJ. Methods and concepts in detecting abnormal reproductive outcomes of paternal origin. Reprod Toxicol. 7:3-16, 1993.

5. Guttenbach M, Engel W, and Schmid M. Analysis of structural and numerical chromosome abnormalities in sperm of normal men and carriers of constitutional chromosome aberrations. A review. Human Genet. 100:1-21, 1997.

6. Hassold T, Abruzzo M, Adkins K, Griffin D, Merrill M, Millie E, Saker D, Shen J, and Zaragoza M. Human aneuploidy: incidence, origin, and etiology. Environ Mol Mutagen. 28:167-175, 1996.

7. Hassold T, Hunt PA, and Sherman S. Trisomy in humans: incidence, origin and etiology. Curr Opin Genet Develop. 3:398-403, 1993.

8. Hassold TJ. Nondisjunction in the human male. Curr Top Develop Biol. 37:383-406, 1998.

9. Olson SB and Magenis RE. Preferential paternal origin of de novo structural chromosome rearrangements. In: The Cytogenetics of Mammalian Autosomal Rearrangements, (A. Daniel, ed. New York:Liss, 1988;583-599.

10. Rudak E, Jacobs PA, and Yanagimachi R. Direct analysis of the chromosome constitution of human spermatozoa. Nature. 274:911-913, 1978.

11. Brandriff B, Gordon L, Ashworth L, Watchmaker G, Carrano A, and Wyrobek A. Chromosomal abnormalities in human sperm: comparisons among four healthy men. Human Genet. 66:193-201, 1984.

12. Brandriff B, Gordon L, Ashworth L, Watchmaker G, Moore D, Wyrobek AJ, and Carrano AV. Chromosomes of human sperm: variability among normal individuals. Human Genet. 70:18-24, 1985.

13. Brandriff BF, Gordon LA, Moore DI, and Carrano AV. An analysis of structural aberrations in human sperm chromosomes. Cytogenet Cell Genet. 47:29-36, 1988.

14. Kamiguchi Y and Mikamo K. An improved, efficient method for analyzing human sperm chromosomes using zona-free hamster ova. Am J Hum Genet. 38:724-740, 1986.

15. Templado C, Benet J, Genesca A, Navarro J, Caballin MR, Miro R, and Egozcue J. Human sperm chromosomes. Hum Reprod. 3:133-138, 1988.

16. Estop AM, Cieply K, Vankirk V, Munn S, and Carver K. Cytogenetic studies in human sperm. Human Genet. 87:447-451, 1991.

17. Estop AM, Marquez C, Munne S, Navarro J, Cieply K, Van Kirk V, Martorell MR, Benet J, and Templado C. An analysis of human sperm chromosome breakpoints. Am J Hum Genet. 56:452-460, 1995.

18. Rosenbusch B, Strehler E, and Sterzik K. Cytogenetics of human spermatozoa: correlations with sperm morphology and age of fertile men. Fertil Steril. 58:1071-1072, 1992.

19. Martin R, Rademaker A, Hildebrand K, Long-Simpson L, Peterson D, and Yamamoto J. Variation in the frequency and type of sperm chromosomal abnormalities among normal men. Human Genet. 77:108-114, 1987.

20. Genesca A, Miro R, Caballin MR, J. B, Germa JR, and Egozcue J. Sperm chromosome studies in individuals treated for testicular cancer. Hum Reprod. 5:286-290, 1990.

21. Robbins W. Cytogenetic damage measured in human sperm following cancer chemotherapy. Mutat Res. 355:235-252, 1996.

22. Martin RH, Hildebrand K, Yamamoto J, Rademaker A, Barnes M, Douglas G, Arthur K, Ringrose T, and Brown IS. An increased frequency of human sperm chromosomal abnormalities after radiotherapy. Mutat Res. 174:219-225, 1986.

23. Martin R, Ernst S, Rademaker A, Barclay L, Ko E, and Summers N. Analysis of sperm chromosome complements before, during, and after chemotherapy. Cancer Genet Cytogenet. 108:133-136, 1999.

24. Robbins WA, Vine MF, Young Truong K, and Everson RB. 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. 30:175-183, 1997.

25. Van Hummelen P, Lowe XR, and Wyrobek AJ. Simultaneous detection of structural and numerical chromosome abnormalities in sperm of healthy men by multicolor fluorescence in situ hybridization. Human Genet. 98:608-615, 1996.

26. Van Hummelen P, Manchester D, Lowe X, and Wyrobek AJ. Meiotic segregation, recombination, and gamete aneuploidy assessed in a t(1;10)(p22.1;q22.3) reciprocal translocation carrier by three- and four probe multicolor FISH in sperm. Am J Hum Genet. 61:651-659, 1997.

27. Sloter E, Lowe X, Moore DI, Nath J, and Wyrobek AJ. Multicolor FISH analysis of chromosomal breaks, duplications, deletions, and numerical abnormalities in the sperm of healthy men. Am J Hum Genet. 67:862-872, 2000.

28. Eastmond DA, Rupa DS, and Hasegawa LS. Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence in situ hybridization with DNA probes. Mutat Res. 322:9-20, 1994.

29. Van Dyke DL, Weiss L, Roberson JR, and Babu VR. The frequency and mutation rate of balanced autosomal rearrangements in man estimated from prenatal genetic studies for advanced maternal age. Am J Hum Genet. 35:301-308, 1983.

30. Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, Summers AM, Meschino WS, Anyane-Yeboa K, Kashork CD, and Reiz KB. Molecular mechanism for duplication 17p11.2- the homologous recombination reciprocal of the Smith-Magenis microdeletion. Nature Genet. 24:84-87, 2000.

31. Jenderny J, Jacobi M, Ruger A, and Rohrborn G. Chromosome aberrations in 450 sperm complements from eight controls and lack of increase after chemotherapy in two patients. Human Genet. 90:151-154, 1992.

32. Sonta S, Yamada M, and Tsukasaki M. Failure of chromosomally abnormal sperm to participate in fertilization in the Chinese hamster. Cytogenet Cell Genet. 57:200-203, 1991.

33. Chayko CA and Martin-DeLeon PA. The murine Rb(6.16) translocation: alterations in the proportion of alternate sperm segregants effecting fertilization in vitro and in vivo. Human Genet. 90:79-85, 1992.

34. Honda H, Miharu N, Samura O, He H, and Ohama K. Meiotic segregation analysis of a 14;21 Robertsonian translocation carrier by fluorescence in situ hybridization. Human Genet. 106:188-193, 2000.

35. Griffin DK, Abruzzo MA, Millie EA, Sheean LA, Feingold E, Sherman SL, and Hassold TJ. Non-disjunction in human sperm: evidence for an effect of increasing paternal age. Hum Mol Genet. 4:2227-2232, 1995.

36. Matsuda Y, Yamada T, and Tobari I. Studies on chromosome aberrations in the eggs of mice fertilized in vitro after irradiation. I. Chromosome aberrations induced in sperm after X-irradiation. Mutation Research148113-117, 1985.

37. Robbins WA, Baulch JE, Moore DI, Weier HU, Blakey D, and Wyrobek AJ. Three-probe fluorescence in situ hybridization to assess chromosome X, Y, and 8 aneuploidy in sperm of 14 men from two healthy groups: Evidence for a paternal age effect on sperm aneuploidy. Reprod Fertil Dev. 7:799-809, 1995.

38. Monteil M, Rousseaux S, Chevret E, Pelletier R, Cozzi J, and Sele B. Increased aneuploid frequency in spermatozoa from a Hodgkin's disease patient after chemotherapy and radiotherapy. Cytogenet Cell Genet. 76:134-138, 1997.

39. Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier HU, Cassel MJ, Wilson G, Eskenazi B, and Wyrobek AJ. Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. Nature Genet. 16:74-78, 1997.

40. Rubes J, Lowe X, Moore DI, Perreault S, Slott V, Everson D, Selevan S, and Wyrobek AJ. Cigarette-smoking lifestyle is associated with increased sperm disomy in teenage men. Fertil Steril. 70:715-723, 1998.

41. Hill F, Marchetti F, Liechty M, Bishop J, Hozier J, and Wyrobek AJ. A new FISH assay to detect chromosome structural aberrations in mouse sperm. Environ Mol Mutagen. 35 (suppl. 31):29, 2000.

42. Shelby MD. Selecting chemicals and assays for assessing mammalian germ cell mutagenicity. Mutat Res. 325:159-167, 1996.

43. Witt KL and Bishop JB. Mutagenicity of anticancer drugs in mammalian germ cells. Mutat Res. 355:209-234, 1996.

44. Sega GA. Unscheduled DNA synthesis (DNA repair) in the germ cells of male mice: its role in the study of mammalian mutagenesis. Genetics. 92:349-358, 1979.

45. Russell L. Effects of spermatogenic cell type on quantity and quality of mutations. In: Male-Mediated Developmental Toxicity, (A. Olsham and D. Mattison, eds.), New York:Plenum Press, 1994;37-48.

46. Generoso WM, Cain KT, Krishna M, and Huff SW. Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg. Proc Natl Acad Sci USA. 76:435-437, 1979.

47. Mailhes JB and Marchetti F. Chemically-induced aneuploidy in mammalian oocytes. Mutat Res. 320:87-111, 1994.

48. Tanaka N, Katoh M, and Iwahar S. Formation of chromosome-type aberrations at the first cleavage after MMS treatment in late spermatids of mice. Cytogenet Cell Genet. 31:145-152, 1981.

49. Albanese R. The use of fertilized mouse eggs in detecting potential clastogens. Mutat Res. 97:315-326, 1982.

50. Matsuda Y and Tobari I. Chromosomal analysis in mouse eggs fertilized in vitro with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulphonate (MMS and EMS). Mutat Res. 198:131-144, 1988.

51. Pacchierotti F, Tiveron C, D'Archivio M, Bassani B, Cordelli E, Leter G, and Spanò M. Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic analysis of one-cell zygotes. Mutat Res. 309:273-284, 1994.

52. Matsuda Y, Seki N, Utsugi-Takeuchi T, and Tobari I. X-Ray- and mitomycin C (MMC)-induced chromosome aberrations in spermiogenic germ cells and the repair capacity of mouse eggs for the X-ray and MMC damage. Mutat Res. 211:65-75, 1989.

53. Matsuda Y, Seki N, Utsugi-Takeuchi T, and Tobari I. Change in X-ray sensitivity of mouse eggs from fertilization to the early pronuclear stage, and their repair capacity. Int J Rad Biol. 55:233-256, 1989.

54. McGaughey RW and Chang MC. Meiosis of mouse egg before and after fertilization. J Exp Zool. 170:397-410, 1969.

55. Donahue RP. Cytogenetic analysis of the first cleavage division in mouse embryos. Proc Natl Acad Sci USA. 69:74-77, 1972.

56. Marchetti F, Lowe X, Moore DI, Bishop J, and Wyrobek AJ. Paternally inherited chromosomal structural aberrations detected in mouse first-cleavage zygote metaphases by multicolor fluorescence in situ hybridization painting. Chromosome Res. 4:604-613, 1996.

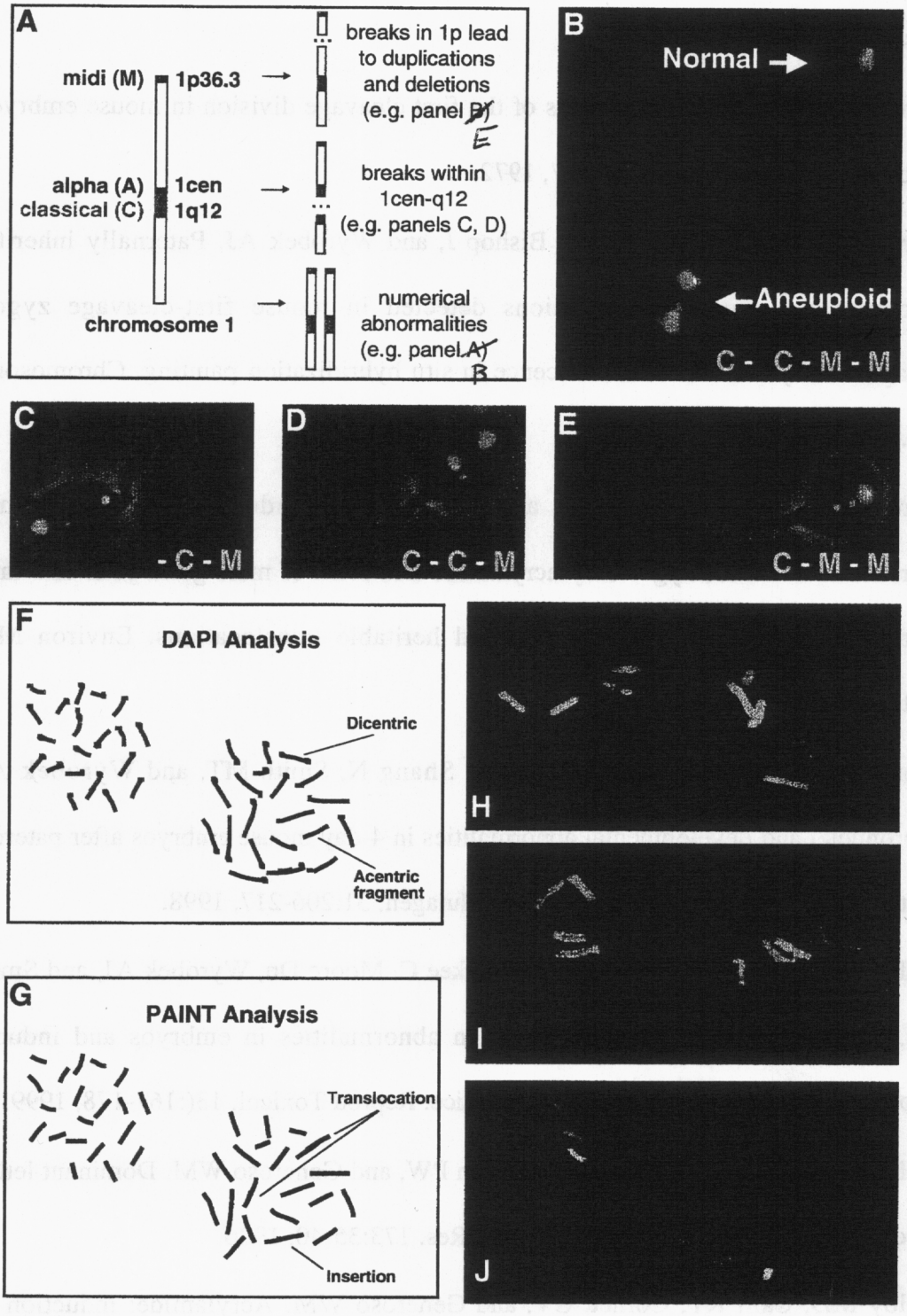
57. Marchetti F, Lowe X, Bishop J, and Wyrobek AJ. Induction of chromosomal aberrations in mouse zygotes by acrylamide treatment of male germ cells and their correlation with dominant lethality and heritable translocations. Environ Mol Mutagen. 30:410-417, 1997.

58. Titenko-Holland N, Ahlborn T, Lowe X, Shang N, Smith MT, and Wyrobek AJ. Micronuclei and developmental abnormalities in 4-day mouse embryos after paternal treatment with acrylamide. Environ Mol Mutagen. 31:206-217, 1998.

59. Holland N, Ahlborn T, Turteltaub K, Markee C, Moore Dn, Wyrobek AJ, and Smith MT. Acrylamide causes preimplantation abnormalities in embryos and induces chromatin-adducts in male germ cells of mice. Reprod Toxicol. 13(:167-178, 1999.

60. Shelby MD, Cain KT, Hughes LA, Braden PW, and Generoso WM. Dominant lethal effects of acrylamide in male mice. Mutat Res. 173:35-40, 1986.

61. Shelby MD, Cain KT, Cornett CV, and Generoso WM. Acrylamide: induction of heritable translocations in male mice. Environ Mol Mutagen. 9:363-368, 1987.


A
midi (M)
1p36.3
alpha (A)
classical (C)
1cen
1q12
chromosome 1
breaks in 1p lead to duplications and deletions (e.g. panel E)
breaks within 1cen-q12 (e.g. panels C, D)
numerical abnormalities (e.g. panel B)
B
Normal
Aneuploid
C - C - M - M
C
- C - M
D
C - C - M
E
C - M - M
F
DAPI Analysis
Dicentric
Acentric fragment
G
PAINT Analysis
Translocation
Insertion
H
I
J

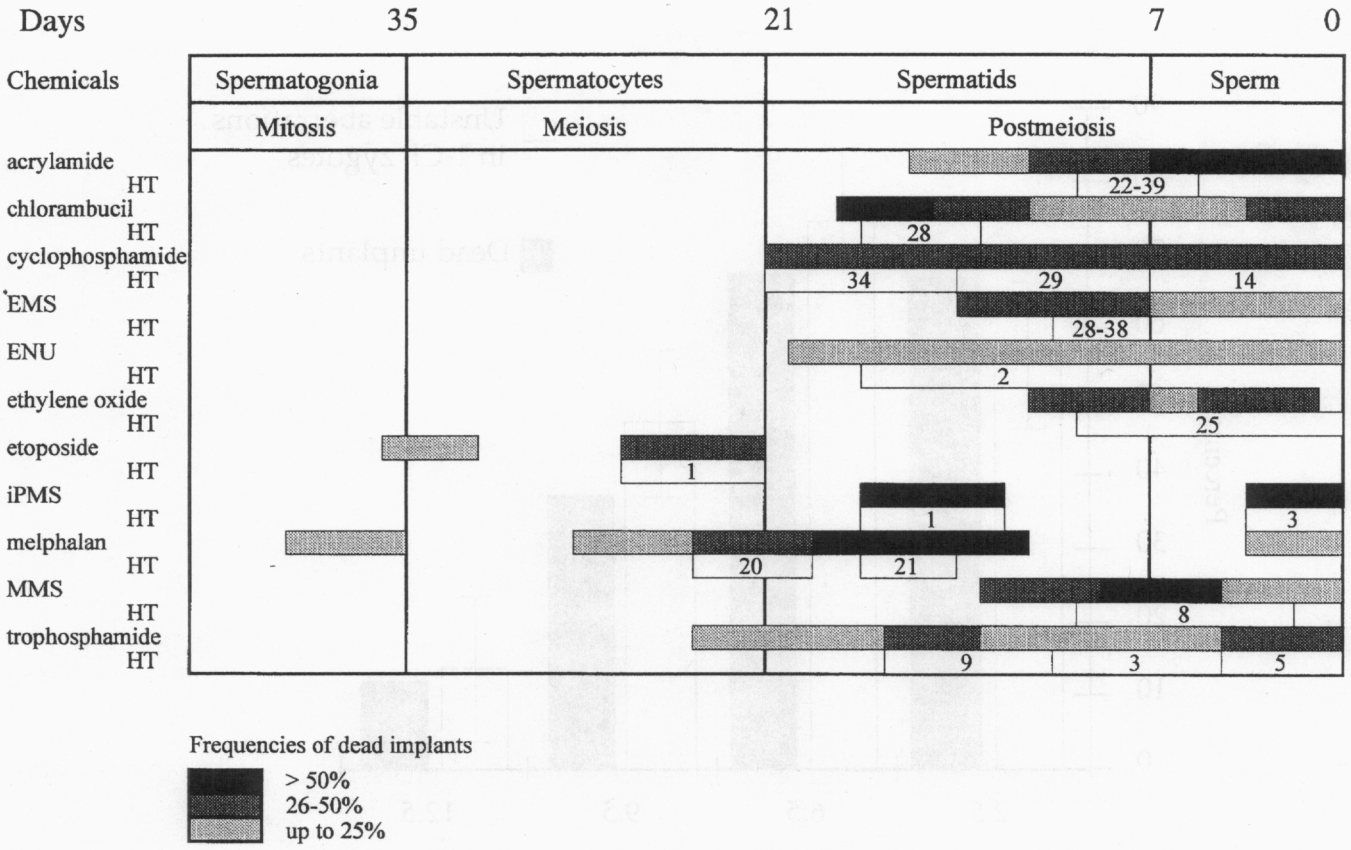

Days
35
21
7
0
Chemicals
Spermatogonia
Spermatocytes
Spermatids
Sperm
Mitosis
Meiosis
Postmeiosis
acrylamide
HT
chlorambucil
HT
cyclophosphamide
HT
EMS
HT
ENU
HT
ethylene oxide
HT
etoposide
HT
iPMS
HT
melphalan
HT
MMS
HT
trophosphamide
HT
22-39
28
34
29
14
28-38
2
25
1
1
3
20
21
8
9
3
5
Frequencies of dead implants
> 50%
26-50%
up to 25%

Figure 2

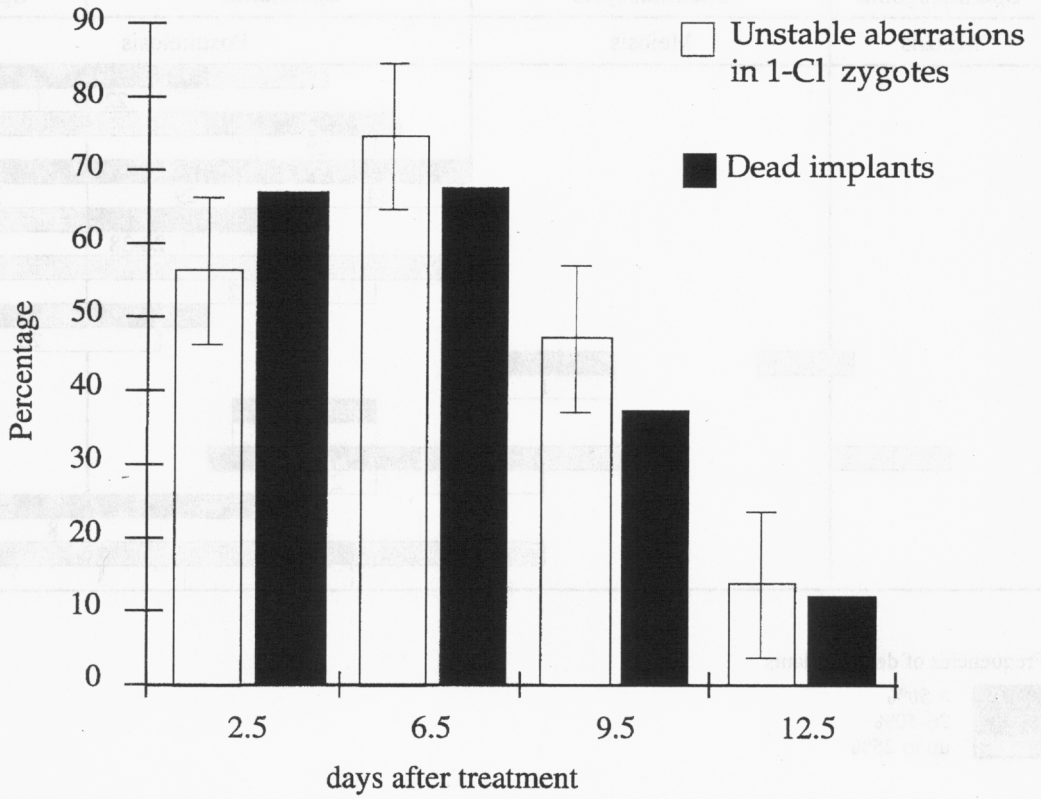

90
80
70
60
50
40
30
20
10
0
Percentage
Unstable aberrations in 1-Cl zygotes
Dead implants
2.5
6.5
9.5
12.5
days after treatment

Figure 3