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# Detection of Nondisjunction in Mammals

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**Methods have been developed in the past to assess spontaneous and induced chromosomal aneuploidy in germ cells and in early pre- and postimplantation mammalian embryos. Some of these methods yield still more information when combined with chromosome banding techniques. Various chemicals and x-rays have been tested in mammalian oogenesis and x-rays in spermatogenesis.**

**The inference may be drawn from these studies that spontaneous nondisjunction is considered to occur only rarely in mouse and hamster oogenesis and spermatogenesis. X-rays induce nondisjunction during male and female meiosis, thus giving rise to significantly more aneuploid oocytes and $F_1$ embryos. The alkylating agents trenimone and cyclophosphamide induce chromosomal missegregation in oocytes; the incidence depends on the dose injected. Hormones used as oral contraceptives did cause aneuploidy in oocytes, but only after daily treatment with high doses. Hormones used for stimulated ovulation did not interfere with chromosome segregation in the mouse and Chinese and Syrian hamsters.**

**The following problems may be considered in future studies: the problem of a species-specificity for induced nondisjunction; the question of a stage sensitivity (transplacental treatment); what happens after chronic exposure, also at low doses; the presence of a threshold; the existence of a dose-effect relation; the nature of cellular target(s) responsible for induced nondisjunction (spindle, regulatory proteins for polymerization of microtubules and their depolymerization, centrioles, centromeres, RNA, or gene expression); whether DNA is involved and whether repair capacity plays a role.**

## Introduction

Aneuploidy represents the most frequent type of chromosomal imbalance in man (*1, 2*) and originates as a rule from chromosomal missegregation during germ cell development. The mechanisms responsible for nondisjunction (ND) during male or female meiosis are only poorly understood, although information has accumulated in the past that chromosomal missegregation during maternal meiosis is the main source of trisomy (*3-5*). It is known furthermore that advanced maternal age increases the risk for trisomy in newborns (6), and there are also hints that medical or occupational x-ray exposure may increase the probability for trisomies among abortions or newborn babies (*7-9*). Although it seems from these studies mentioned above that oogenesis is more prone to chromosomal malsegregation it was shown indeed, that failures from spermatogenesis may contribute as well to trisomy among newborns (*3, 4*).

Experiments with mammals made us aware that exposure to x-rays and to some chemicals, like alkylating agents, antimetabolites, cadmium, and some hormones induces ND in germ cells and so is potentially harmful in increasing aneuploidy in man. Some of these experiments on mutagen-induced ND in mammalian germ cells will be discussed here briefly, without the intention of giving a complete review on this topic.

## Nondisjunction in Oogenesis

Methods have been developed in the past and are now available for cytogenetic studies to assess meiotic nondisjunction at various stages of development. The first studies are possible in ovulated oocytes where aneuploidy is measurable immediately after meiosis I (*10*) or in preimplantation embryos (*11-13*), from pronuclei up to blastocysts, as well as in postimplantation embryos (*14, 15*). The methods now commonly used are basically techniques adopted from Tarkowsky's air drying technique (*16*) for oocytes and early embryos and the method of Evans et al. (*17*) for postimplantation embryos.

The most commonly used species in these studies so far is the mouse. Recently, however, the Chinese hamster (*18, 19*), the Syrian hamster (*20, 21*), and the

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Djungarian hamster have been adopted for these purposes; these are most useful for questions such as a species-specificity or to study the question of a higher susceptibility of specific chromosomes, e.g. NOR-bearing chromosomes, acrocentric, etc., for missegregation.

It is known now that the incidence of aneuploidy due to meiotic nondisjunction, without any exposure to mutagens, is very low in the mammals tested so far and hence differ significantly from the estimated incidence in man (Table 1). The data for oocytes from mice (*2*) and Chinese (*21*), Syrian (*20, 21*), and Djungarian hamsters point to an incidence of first meiotic cleavage errors below 1%. This frequency may increase to approximately 1% early after fertilization due to errors during maternal meiosis II and paternal meiosis I/II (*13*), but decreases again during the following days in a response to elimination with respect to aneuploid embryos.

Aneuploidies in embryos originate, as already mentioned, from nondisjunction of whole chromosomes during meiosis (the possibility of postmeiotic nondisjunction resulting in mosaicism is not considered here). We do have evidence that also presegregation during meiosis I may potentially contribute to the incidence of trisomy and monosomy. Among 3338 analyzed mouse oocytes we found nine in which a single chromatid was absent (19 chromosomes plus one single chromatid) and another six with an extra chromatid (20 chromosomes plus one single chromatid) (*22*). Destaining and restaining for centromeric heterochromatin revealed that all these chromatids do carry centromeric heterochromatin and possibily do have a functionally intact centromere. These types have been also observed after mutagen exposure (*23*). The process of presegregation seems not to be restricted to mouse oocytes and to acrocentric chromosomes, because single chromatids were also observed in oocytes from Chinese- and Djungarian hamsters after hormonal stimulation or irradiation (Hansmann and Probeck, unpublished data).

## Effect of Hormones on Nondisjunction

The hormones used in several laboratories to induce ovulation, i.e. pregnant mares' serum (PMS) and human chorionic gonadotrophin (HCG)—do not enhance ND during the first meiotic division in oocytes from mouse (*22*), Chinese (*21*), and Syrian hamsters (*20, 21*). Significantly more hyperploid oocytes were ovulated after the injection of high doses of PMS and HCG in Djungarian hamster females (Hansmann and Probeck, unpublished data). The mechanisms involved are still unknown, but the observation underlines the possibility of species-specificity in induced ND.

Hormonally induced ovulation may have also an influence on chromosome segregation in human oocytes (*24*). It was reported that women had more often a chromosomally unbalanced (trisomy, polyploidy) spontaneous abortion after induced ovulation than had women without hormone injection. The risk was also higher in the group of those women who got pregnant within the first cycles after treatment compared to the second group having conception only later.

An early study from Carr (*25*) led to the suggestion that hormones used as oral contraceptives may have an effect on female meiosis. He reported that women getting pregnant soon after stopping the pill had statistically significantly more triploid abortions. His suggestion was, however, not confirmed by later studies with spontaneous abortions (*26, 27*) albeit *in vitro* studies with human oocytes corroborated the finding of polyploidy (*28*).

**Table 1. Incidence of "spontaneous" nondisjunction in oogenesis of several rodents.[a]**

| Species | Aneuploidy assessed in | No. of oocytes or embryos analyzed | No. hyperploid | Incidence of aneuploidy, % (hyperploid × 2) | Reference |
|---|---|---|---|---|---|
| Mouse (*Mus musculus*) | oocytes | 5853 | 28 | 0.48 | (*10, 22, 23, 29, 32-34, 39, 40*) |
| | pronuclei | 2902 | 24 | 1.65 | (*41*) |
| Chinese hamster | oocytes | 455 | 0 | 0 | (*14, 21*) |
| (*Cricetulus griseus*) | 4-8 cell embryos | 226 | 1 | 0.89[b] | (*42*) |
| Syrian hamster (*Mesocricetus auratus*) | oocytes | 455 | 1 | 0.44 | (*20, 21*) |
| Djungarian hamster (*Phodopus sungorus*) | oocytes | 197 | 0 | 0 | Hansmann and Probeck, unpublished |

[a]The data are put together, although different methods have been used (*in vivo*/*in vitro* maturation, hormonal induced ovulation/spontaneous ovulation, different strains, etc.) for the different groups.

[b]One embryo affected by a monosomy was reported by the authors.

We studied the possible influence of such hormones in our mouse system (*29*) and treated C3H females daily for 4 weeks with 0, 1, or 10 mg norethisterone acetate, orally. Chromosomes were analyzed in oocytes from spontaneously ovulating females immediately after treatment (week 1 and 2) or after a longer interval without any treatment (weeks 7, 8, and 9). A statistically significant increase of hyperploid oocytes—a conservative parameter of ND—was observed only after the high, unphysiological dose of the gestagen, but not in the low dose group.

## Effect of Alkylating Agents and Antimetabolites

When injected during the preovulatory phase the alkylating agent trenimone induced ND in (101 × C3H) $F_1$ mouse females (*10*). The lowest tested dose which was effective was 0.25 mg/kg body weight, and this dose resulted in approximately a four-fold increase compared to untreated females when the substance was injected at the same time with HCG. In studies with two-cell stages (*30*) and early morulae (*12*) it was shown that the same kind of treatment of preovulatory oocytes enhanced aneuploidy in early prenatal development.

Subsequent studies with two hamster species led us to assume that one species may not react in the same way as another species after the same kind of treatment. No hyperploidy was detected in 235 oocytes from Syrian hamster females after injection of trenimone (*20*), and only three oocytes with an odd chromosome (1 additional acrocentric chromosome, 2 chromosomes surplus from group 3, 4, or X) in 157 oocytes from Chinese hamster females (*18*).

Cyclophosphamide, the second alkylating compound tested in our laboratory, is also an inducer of aneuploidy in mouse oocytes when injected during the sensitive preovulatory phase. It is not effective, however, when the compound is injected on days 11 and 17 during pregnancy (*11*).

Amethopterin, a folic acid antagonist was tested in several stages of mouse oogenesis (*11*): (1) transplacentally, by injection on days 11, 13, and 17 of gestation, to study the effect on embryonic gonads; (2) by injecting 3-week-old females to test the sensitivity of dictyate oocytes; (3) during the preovulatory phase, i.e. 3 hr after HCG-application; and (4) 8.5 hr after HCG, i.e., after the first meiotic division. No effect was observed in the last group, as could be expected from the observation that meiosis I is already completed at this time. Hyperploid oocytes were only detected after treating the sensitive preovulatory phase and the embryonic gonads, i.e., oogonia/primordial germ cells, on day 11 of gestation. Treatment of the other meiotic stages obviously did not influence ND.

Still more information is required on the sensitivity of different stages of oogenesis.

## Effect of Other Chemicals on Nondisjunction

Trypaflavin, a therapeutically used acridine, was given for 50 days to NMR I mouse females at a daily dose of 2 mg/kg (*31*). Chromosome analysis of ovulated oocytes revealed no significant effect on the incidence of hyperploidy.

Three of 271 oocytes from mouse strain ddy (*32*) had a supernumerary chromosome after treatment with cadmium, compared to none among 198 oocytes of untreated females. It was suggested by the authors, however, that the difference was statistically not significant.

## Effect of X-Rays on Nondisjunction

According to several studies one may accept that irradiation induces nondisjunction in mammalian oocytes, although no effect was yet demonstrated in Chinese hamster females (*14*). This conclusion can be reached considering the studies in mouse oocytes (*33, 34*) and in early postimplantation embryos (*14*) after irradiation females immediately before first meiotic division. The effect of x-rays on aneuploidy was much more pronounced in older females (*35, 36*).

## Conclusions of the Study of Nondisjunction in Mammalian Oogenesis

Nondisjunction is most easily detectable in ovulated oocytes from several mammalian species immediately after meiosis I.

Appropriate stages for the assessment of ND during postmeiotic development are zygotes at the pronucleus stage and early postimplantation embryos. Chromosome banding techniques are used with success at both stages to identify the failure.

Spontaneous ND is lower by several factors in mammals compared to the estimated incidence in man.

Hormones used for stimulated ovulation have no effect on chromosome segregation during the first meiotic division in three species, but do induce ND at high doses in Djungarian hamsters.

Some chemicals — already known to be mutagens — induce nondisjunction in oocytes.

The incidence of ND depends probably on the injected dose of chemical as well as on the stage of meiosis. Sensitive so far is the short phase before ovulation and a phase during early prenatal de-

velopment (oogonia/primordial germ cells).

X-rays incude ND in mouse oocytes, especially in those from older females.

Studies in the future may consider: the problem of species-specificity in mammals; the question of stage sensitivity (transplacental treatment); chronic exposure, also exposure to low doses; the dose-effect relation, i.e., the presence of a threshold; the nature of the cellular target (s) responsible for induced nondisjunction (spindle, regulatory proteins, centromeres, centrioles, RNA, gene-expression); the involvement of DNA and importance of repair.

## Studies in Spermatogenesis

Nondisjunction during the first meiotic division in male mice is indeed a very rare event (*37*) and occurs not as often as in oogenesis. Increased nondisjunction of X and Y chromosomes after exposure to x-rays was shown both by cytological and noncytological methods (*15, 38*).

It was not clear whether irradiation of spermatogenesis would produce an increase of cytogenetically detectable trisomy in the resulting $F_1$ generation. We followed this line and irradiated 10 males with 20 or 200 R and mated them to untreated females for 57 days consecutively. The resulting $F_1$ embryos were prepared cytologically on day 9.5 post coitus (pc) and chromosomes stained with a Trypsin-Leishman banding method. Karyotypes were prepared from each embryo from one metaphase. In cases of an anomaly as many karyotypes as possible were compared. Irradiated fathers produced significantly more aneuploid $F_1$ embryos (*14*, and Hansmann and Probeck, unpublished data) than nonirradiated fathers. A clearcut dose-effect relation could not be shown yet. The $F_1$ embryos, although severely retarded in some cases, had the following trisomy or monosomy on day 9.5 pc (day of vaginal plug is day 0.5): trisomy: 2, 11 (2x), 13 (2x), 14, and also XYY; monosomy: 8, X (3x).

Our study on radiation-induced ND in mouse spermatogenesis underlines an earlier made suggestion of a higher risk for aneuploidy among spontaneous abortions after paternal exposure to x-rays (*7*). The results emphasize the necessity to include spermatogenesis as well in mutagenesis studies on chromosomal missegregation.

The study of early postimplantation embryos is, especially in combination with banding techniques, a very useful tool for the analysis of mutagen-induced meiotic ND and structural anomalies from spermatogenesis and oogenesis. It may be especially useful when both the male and the female population is exposed to mutagens.

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### REFERENCES

1. Nielsen, J., and Sillesen, I. Incidence of chromosome aberrations among 11,148 newborn children. Hum. Genet. 30: 1 (1975).
2. Boué, J., and Boué, A. Les avortements spontanés humains: études cytogénétiques et epidémiologiques. Rev. Franc., Gynec., 68: 625 (1973).
3. Langenbeck, U., Hansmann, I., Hinney, B., and Hönig, V. On the origin of the supernumerary chromosome in autosomal trisomies with special reference to Down's syndrome. A bias in tracing nondisjunction by chromosomal and biochemical polymorphisms. Hum. Genet., 33: 89 (1976).
4. Jacobs, P. A., and Morton, N. M. Origin of human trisomics and polyploids. Hum. Hered., 27: 59 (1977).
5. Hassold, T., and Matsuyama, A. Origin of trisomies in human spontaneous abortions. Hum. Genet., 46: 285 (1979).
6. Penrose, L. S. The relative effects of paternal and maternal age in mongolism. J. Genet., 27: 219 (1933).
7. Lazar, P., Gueguen, S., and Boué, A. Epidémiologie des avortements spontaneés précoces: á propos de 1469 avortements caryotypes. In: Les Accidents chromosomiques de la Réproduction, A. Boué and C. Thibault, Eds., INSERM Paris, 1973.
8. Alberman, E., Polani, P. E., Fraser Roberts, J. A., Spicer, C. C., Elliot, M., Armstrong, E., and Dhadial, R. K. Parental x-irradiation and chromosome constitution in their spontaneously aborted foetuses. Ann. Hum. Genet., 36: 185 (1972).
9. Uchida, I. Maternal radiation and trisomy, 21. In: Population Cytogenetic Studies in Humans. E. B. Hook and I. H. Porter, Eds., Academic Press, New York, 1977.
10. Röhrborn, G., and Hansmann, I., Induced chromosome aberrations in unfertilized oocytes of mice. Hum. Genet., 13: 184 (1971).
11. Hansmann, I. Induced chromosomal aberrations in pronuclei, 2-cell stages and morulae of mice. Mutat. Res., 20: 353 (1973).
12. Hansmann, I., and Röhrborn, G. Chromosome aberrations in preimplantation stages of mice after treatment with triazoquinone. Hum. Genet., 18: 101 (1973).
13. Maudlin, I., and Fraser, L. R. The effect of PMSG dose on the incidence of chromosomal anomalies in mouse embryos fertilized *in vitro*. J. Reprod. Fert., 50: 275 (1977).
14. Hansmann, I. The induction of nondisjunction by irradiation in mammalian oogenesis and spermatogenesis. In: Proceedings of the Meeting of C.E.C. Contractants on the Genetic Effects of Radiation, Brussels, 1979, Mutat. Res., in press.
15. Szemere, G., and Chandley, A. Trisomy and triploidy induced by x-irradiation of mouse spermatocytes. Mutat. Res., 33: 229 (1975).
16. Tarkowsky, A. K. An air drying method for chromosome preparations from mouse eggs. Cytogenet. Cell Genet., 5: 394 (1966).
17. Evans, E. P., Burtenshaw, M. D., and Ford, C. E. Chromosomes of mouse embryos and newborn young: Preparation from membranes and tail tips. Stain Technol. 47: 229 (1972).
18. Hansmann, I., Neher, J., Röhrborn, G. Chromosome aberrations in metaphase II oocytes of Chinese hamster (*Cricetulus griseus*). I. The sensitivity of the preovulatory phase to triaziquone. Mutat. Res., 25: 347 (1974).
19. Binkert, F., and Schmid, W. Preimplantation embryos of

hamster. II. Incidence and type of karyotype anomalies after treatment of the paternal postmeiotic germ cells with an alkylating mutagen. Mutat. Res., 46: 77 (1977).
20. Basler, A. Timing of meiotic stages in oocytes of the Syrian hamster (*Mesocricetus auratus*) and analysis of induced chromosome aberrations. Hum. Genet., 42, 67 (1978).
21. Hansmann, I., and Probeck, H. D. Chromosomal imbalance in ovulated oocytes from Syrian hamsters (*Mesocricetus auratus*) and Chinese hamsters (*Cricetulus griseus*). Cytogenet. Cell Genet., 23: 70 (1979).
22. Hansmann, I., and El Nahass, E. Incidence of nondisjunction in mouse oocytes. Cytogenet. Cell Genet., in press.
23. Hansmann, I. Chromosome aberrations in metaphase II-oocytes. Stage sensitivity in mouse oogenesis to amethopterin and cyclophosphamide. Mutat. Res. 22: 175 (1974).
24. Boué, J., and Boué, A. Increased frequency of chromosomal anomalies in abortions after induced ovulation. Lancet I: 679 (1973).
25. Carr, D. H. Chromosomes after oral contraceptives. Lancet II: 830 (1967).
26. Boué, A. and Boué, J. Études chromosomiques et anatomiques des grossesses suivant l'arrêt de contraceptifs steroides. J. Gyn. Oost. Biol. Répr., 2: 141 (1973).
27. Therkelsen, A. J., Grummet, N., Hjort, T., Jensen, O. M., Jonasson, J., Lauritsen, J. G., Lindsten, J., and Petersen, B. Studies on spontaneous abortions. In: Comptes Rendus du Colloque sur les Accidents Chromosomiques de la Réproduction, A. Boué and C. Thibault, Eds., INSERM, Paris, 1973.
28. Klinger, H., Kawa, H. W., Glasser, M., and Kallenberg, A. Cytogenetic anthropometric and developmental studies of post-contraceptive reproduction in man. In: Comptes Rendus du Colloque sur les Accidents Chromosomiques de la Réproduction. A. Boué and C. Thibault, Eds., INSERM, Paris, 1973.
29. Röhrborn, G., and Hansmann, I. Oral contraceptives and chromosome segregation in oocytes of mice. Mutat Res. 26: 535 (1974).
30. Röhrborn, G., Kühn, O., Hansmann, I., and Thon, K. Induced chromosome aberrations in early embryogenesis of mice. Hum. Genet., 11: 316 (1971).
31. Basler, A., Brucklacher, M., Nobis, F., and Röhrborn, G. Comparative investigations with trypaflavin in metaphase-II oocytes and in dominant lethal assay. Hum. Genet., 40: 87 (1977).
32. Shimada, T., Watanabe, T., and Endo, A. Potential mutagenicity of cadmium in mammalian oocytes. Mutat. Res. 40: 389 (1976).
33. Uchida, I., and Lee, C. P. V. Radiation induced nondisjunction in mouse oocytes. Nature 250: 601 (1974).
34. Reichert, W., Hansmann, I., and Röhrborn, G. Chromosome anomalies in mouse oocytes after irradiation. Hum. Genet. 28: 25 (1975).
35. Yamamoto, M., Shimada, T., Endo, A., and Watanabe, G. Effects of low-dose irradiation on the chromosomal nondisjunction in aged mice. Nature New Biol., 244: 206 (1973).
36. Uchida, I. A., and Freeman, C. P. V. Radiation-induced nondisjunction in oocytes of aged mice. Nature 265: 186 (1977).
37. Ohno, S., Kaplan, W. D., and Kinosita, R. Do XY and O sperm occur in *Mus musculus*. Exptl. Cell Res. 18: 382 (1959).
38. Russell, L. B., and Saylors, C. L. The relative sensitivity of various germ-cell stages of the mouse to radiation-induced nondisjunction chromosome-losses and deficiencies. In: Repair from Genetic Radiation Damage, F. H. Sobels, Ed., Pergamon Press, Oxford, 1953.
39. Martin, R. H., Dill, F. J., and Miller, J. R. Nondisjunction in aging female mice. Cytogenet. Cell Genet., 17: 150 (1976).
40. Becker, K., and Schöneich, J. Frequencies of spontaneous meiotic nondisjunction in mouse oocytes. Paper presented at 6th Annual Meeting, EEMS, Gernrode, 1976.
41. Maudlin, I., and Fraser, L. R. The effect of sperm and egg genotype on the incidence of chromosomal anomalies in mouse embryos fertilized *in vitro* J. Reprod. Fert., 52: 107 (1978).
42. Binkert, F., and Schmid, W. Preimplantation embryos of Chinese hamster. I. Incidence of karyotype anomalies in 226 control embryos. Mutat. Res. 46: 63 (1977).