

# A cytogenetic study of G1-chromosomes in one-cell stage mouse embryo and in corresponding second polar body. Evaluation of aneuploidy originated in females heterozygous for translocation T[14;15]6Ca

ANDREI DYBAN<sup>1\*</sup>, MICHAEL FREIDINE<sup>2</sup> and EKATERINA NONIASHVILI<sup>1</sup>

<sup>1</sup>Institute for Experimental Medicine, Russian Academy of Medical Sciences, Sankt Petersburg, Russia and <sup>2</sup>Reproductive Genetics Institute, Chicago, Illinois, USA

**ABSTRACT** Meiotic chromosomes in ovulated oocytes and G1-chromosome sets visualized in the 2nd PB and in the pronuclei of one-cell stage embryos treated with okadaic acid were studied in female mouse heterozygous for reciprocal translocation T[14;15]6Ca. It was found that 61.5% of oocytes were haploid, 14.9% hyperhaploid and 23.6% hypohaploid. Unpaired chromatid (a half-dyad), in addition to (or replacing) a whole chromosome (a dyad), was detected in 20% of oocytes. G1-chromosome complements in the 2nd PB and in the MPN expected in the case of aneuploidy due to chromosome non-disjunction or to chromatid abnormal segregation at the first and second meiotic division were detected in one-cell stage embryos. The hypo- and hyperhaploidy caused by non-disjunction was revealed in 17.6% of embryos. Aneuploidy due to abnormal segregation of a chromatid in the first and the second meiotic division was in 20% and 4.4% of all cases respectively. The incidence of different classes of aneuploid oocytes were almost fully conformable to that of corresponding types of aneuploidy detected in one-cell stage embryos. The main advantage of the proposed new approach based on cytogenetic analysis of G1-chromosomes in the 2nd PB and in the corresponding MPN is that it allows to study not only the chromosome non-disjunction, but also abnormal segregation of chromatids in the first and in the second meiotic divisions, and to estimate accurately the incidence of these maternal meiotic errors.

**KEY WORDS:** *meiosis, G1-chromosomes, polar body, pronucleus*

## Introduction

Human aneuploidy is one of the major components of genetic abnormalities, which eventually leads to reproductive failure, spontaneous abortions, congenital malformations physical abnormalities and mental retardation. The literature on aneuploidy has become enormous, and many monographs devoted to clinical and experimental aspects of human aneuploidy have been published (for references see Gaulden, 1992). Although aneuploidy can arise in the spermatogenesis and oogenesis either at the first or the second meiotic divisions, as well as during cleavage, it is a well known fact that in humans a vast majority of numerical chromosomal aberrations originate from maternal errors at the first meiotic division (Hassold *et al.*, 1991; Antonarakis *et al.*, 1992). It was shown that human trisomy and monosomy emerged in oogenesis not only as the result of chromosome nondisjunction (for review see Bond and Chandley, 1983; Hansmann, 1983; Chandley, 1987; Dyban *et al.*, 1993 b; Kola *et al.*, 1993), but as a consequence of

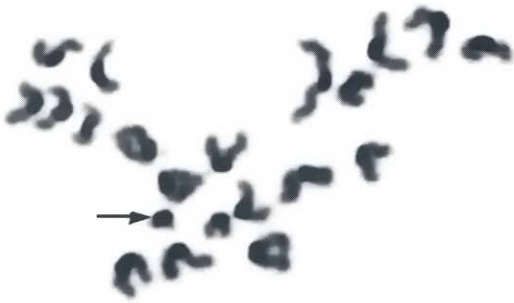
a chromosome loss following anaphase lagging (Chandley, 1987). It was proposed that human trisomy derived merely from abnormal segregation of an unpartnered chromatid at the first meiotic division of oocytes (Angell, 1991; Angell *et al.*, 1993, 1994). Many hypotheses on the etiology of the maternal meiotic errors are formulated (for review see Gaulden, 1992), but the problem remains unsolved.

The difficulties for the study of the causes of aneuploidy in humans can be avoided by the use of experimental animals, and the mouse has been widely utilized for this purpose. Different chemicals (for review see Pacchierotti, 1988; Mailhes and Marchetti, 1994) and radiation (Tease, 1988 and references therein) were

*Abbreviations used in this paper:* MII, metaphase of the second meiotic division; 1st PB, first polar body; 2nd PB, second polar body; MPN, maternal pronucleus; PPN, paternal pronucleus; OA, okadaic acid; T6/+, mouse heterozygous for reciprocal translocation T[14;15]6Ca; G1-chromosomes, prematurely condensed chromosomes at the G1-phase of cell cycle.

\*Address for reprints: Department of Molecular Genetics, Institute for Experimental Medicine, Pavlov St. 12, Sankt Petersburg, 197376, Russia. FAX: 7(812)-234-9489 and 7(812)-245-4586. e-mail: dyban@AD1579.spb.edu





**Fig. 1. Hyperhaploid second meiotic metaphase in T6/+ oocyte with 21 whole chromosomes (dyads).** The small marker chromosome  $15^{14}$  is clearly seen (arrow). C-banding with silver, counter-staining with Giemsa. (x 1250).

tested on female germ cell, but in these studies attention was generally focused on hyperhaploid oocytes and zygotes. Hypohaploid chromosome complements supposed to be the result of technical artifacts were discarded, and the rate of aneuploidy was estimated by doubling the rate of hyperhaploidy. It should be mentioned that omitting hypohaploidy from the calculation of the rate of aneuploidy disregards the possibility of non-random chromosome segregation (for references see Sakurada *et al.*, 1994), a chromosome loss following anaphase lagging and abnormal segregation of an unpartnered chromatid during the first meiotic division. Therefore further studies of the action of environmental agents on female gametes are needed, and the results of these experiments would strongly depend on the improvement of the technique used for detecting maternal meiotic errors.

A long time ago it was suggested that the cytogenetic analysis of the second polar body (2nd PB) can substantially contribute to the study of aneuploidy which emerged during oogenesis (Modlinski and McLaren, 1980, McLaren, 1985). However, in the mouse, like in many other mammalian species, the 2nd PB never enters mitosis. Taking this into account, the 2nd PB was transplanted into a fertilized oocyte and it was shown that in the cytoplasm of zygote the nucleus of the 2nd PB was transformed to a presumably haploid metaphase (Modlinski and McLaren, 1980). Recently these observations were confirmed by electrofusion of the mouse 2nd PB with normal or enucleated zygote, and the suggestion was made of this technique being applicable in the preimplantation diagnosis based on a karyotype of the isolated 2nd PB (Yerlinsky *et al.*, 1994; Evsikov and Evsikov, 1995).

The other approach, which is easier and quicker, is based on the reaction of a cell nucleus on okadaic acid, a specific inhibitor of phosphoprotein phosphatases 1 and 2 A (for review see Cohen, 1989, Cohen *et al.*, 1990, Schonthal, 1992). It was shown that short incubation of one-cell stage mouse embryos in a culture medium with okadaic acid (OA) allows to visualize prematurely condensed interphase chromosomes (PCC) in both pronuclei and in the corresponding 2nd PB. It was proven that after OA treatment it is possible to detect in chromosome spreads the marker chromosome and to count the number of chromosomes in the 2nd PB, as well as in the pronuclei (Dyban *et al.*, 1992, 1993a).

Our goal was to answer the question, using this approach, whether the comparison of G1-chromosome complements in the 2nd PB and in the MPN allows to reveal the main types of aneuploidy emerged in oocytes at the first and the second meiotic divisions. The study was designed to determine the advantages

and the limitations of this new cytogenetic assay of maternal meiotic errors, and our experiments were deliberately performed on female mice with a known incidence of aneuploidy. Used in the experiments were heterozygous for reciprocal translocation T[14;15]6Ca females with a high rate of non-disjunction (Oshimura and Takagi, 1975; Kaufman, 1976; Dyban and Baranov, 1987), and with unpartnered chromatids in MII oocytes (Oshimura and Takagi, 1975).

## Results

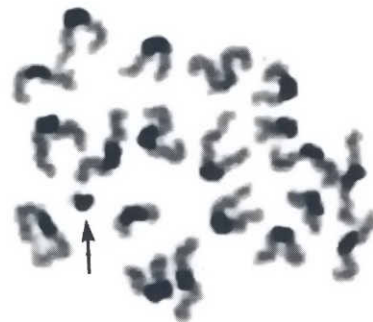
### Cytogenetic analysis of ovulated oocytes

It should be pointed out that C-banding with silver staining permitted not only to count exactly the number of chromosomes, but allowed, in addition, recognition of the small marker T6 chromosome  $15^{14}$  and the unpaired chromatid (Figs. 1, 2) The results of chromosome observations in 470 secondary T6/+ oocytes are summarized in Table 1. Well-prepared chromosome spreads were found in 390 oocytes, 240 oocytes (61.5%) were euploid and 150 oocytes (38.5%) contained aneuploid set of chromosomes. Hyperhaploidy was found in 58 oocytes (14.9%) and hypohaploidy was registered in 92 oocytes (23.6%). Thirty hyperhaploid metaphases (7.7%) were with 21 chromosomes (Fig 1), and 40 hypohaploid oocytes (10.9%) contained 19 chromosomes. It is noticeable that in 27 hyperhaploid oocytes (6.9%) one extra unpartnered chromatid (a half-dyad) was detected (Fig. 2). The karyotype of these oocytes was  $20+1C$  and the total number of chromatids equalled 41. In 50 hypohaploid oocytes (12.8%) 19 chromosomes and one unpaired chromatid were present, i.e., they possessed 39 chromatids and the karyotype  $19+1C$ . It deserves mention that in all hyper or hypohaploid metaphases, in which a single chromatid was found, the unpartnered chromatid belongs to a small T6 marker chromosome  $15^{14}$  (Fig. 2).

### Cytogenetic analysis of one-cell stage embryos and corresponding second polar bodies.

#### Predicted G1-chromosome complements in the second polar body and in the maternal pronucleus.

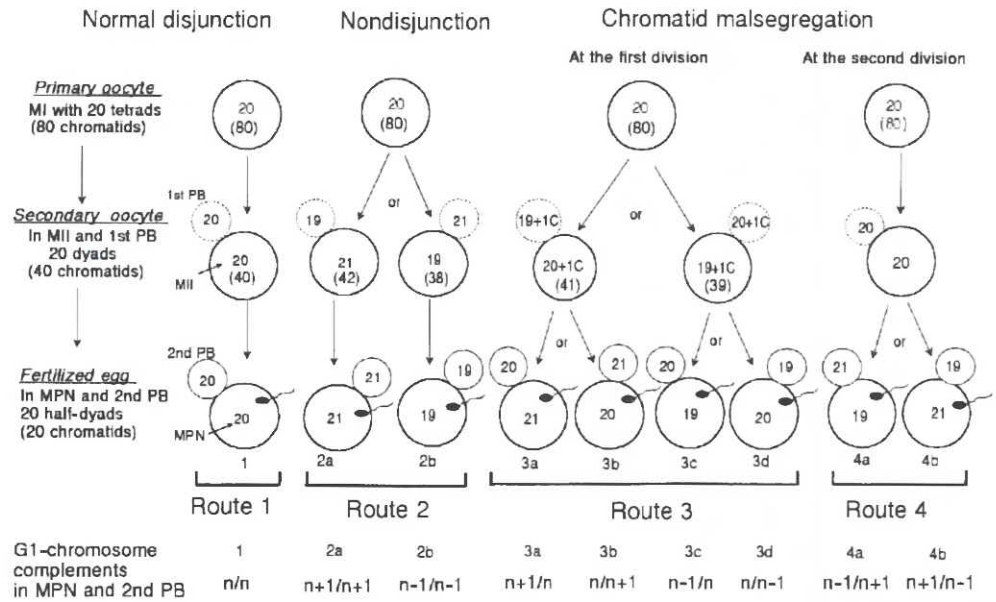
It is a well known fact that when in the oogenesis the first meiotic division errors are followed by abnormal segregation of chromosomes in the second meiotic division, a vast variety of combinations of chromosome complements in the cleavage mitosis may



**Fig. 2. Hyperhaploid second meiotic metaphase in T6/+ oocyte with 20 whole chromosomes and one unpartnered chromatid (arrow).** C-banding with silver, counter-staining with Giemsa. (x 1250).



**Fig. 3. Diagram of the variants of G1-chromosome complements in the maternal pronuclei and in the corresponding second polar bodies.** They should be expected in the case of aneuploidy due to chromosome nondisjunction or abnormal segregation of unpaired chromatid at the first meiotic division or after abnormal segregation at the second meiotic division. Abbreviations: MI, metaphase of the first meiotic division; MII, metaphase of the second meiotic division; 1st PB, first polar body; 2nd PB, second polar body; MPN, maternal pronucleus; 1C, one unpartnered chromatid.



emerge. Abnormalities of this kind, however, are infrequent and in Figure 3 only the main variants of possible G1-chromosome complements that should emerge in the 2nd PB and in the corresponding MPN are presented. Normal disjunction (Fig. 3, route 1) must lead to the formation of euploid and haploid complements in the MPN and in the 2nd PB. When at the first meiotic division non-disjunction of a whole chromosome (a dyad) occurs, the MPN and the corresponding 2nd PB would have the same aneuploid number of chromosomes, i.e., n+1 and n+1 (Fig. 3, route 2a) or n-1 and n-1 (Fig. 3, route 2b). In the case of true hypohaploidy both sets of chromosomes must be hypohaploid (n-1), lacking the same chromosome. Segregation of an unpartnered chromatid into the secondary oocyte would lead to hyperhaploidy (n+1) in the MPN, accompanied by the formation of an euploid and haploid set in the 2nd PB (Fig 3, route 3a), or vice versa (Fig. 3, route 3b), while the absence of one chromatid in the secondary oocyte (the set 19+1C) must lead to hypohaploidy in the MPN (n=19) and the number of chromosomes in the 2nd PB would be euploid and

haploid (Fig. 3, route 3c), or vice versa (Fig. 3 route 3d). Abnormal segregation in the second meiotic division would cause the formation of complementary sets, i.e., hyperhaploidy (n+1) in the 2nd PB and hypohaploidy (n-1) in the MPN (Fig 3, route 4a), and vice versa. (Fig 3, route 4b). In both cases the sum of chromosomes in the MPN and in the 2nd PB must be euploid and diploid, i.e. equals 40.

*The detected G1-chromosome complements in the embryos and in the second polar bodies*

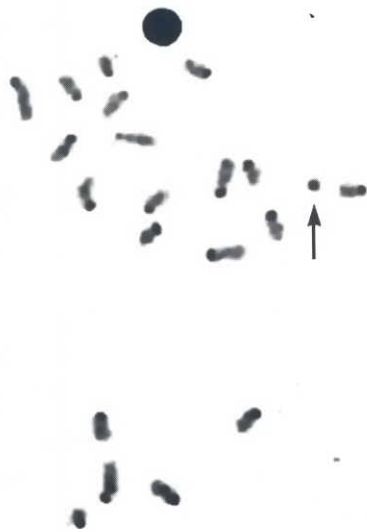
After the action of OA on the one-cell stage embryo the PCC were visualized in the pronuclei and in the 2nd PB. In some cases the S-type of PCC was found, but in a large proportion of embryos the G1 type of PCC were present. For the 2nd PB noticeable were more contracted chromosomes, which frequently were not co-oriented, but irregularly scattered. The G1-chromosomes in the paternal pronucleus (PPN) were decidedly longer than in the MPN, whereas those derived from the MPN were regularly organized and longer than in the 2nd PB nucleus. In some plates, originated from

TABLE 1

**FREQUENCIES OF VARIOUS CLASSES OF T6/+ OOCYTES**

Total No. of analyzed MII oocytes	No. of MII oocytes with analyzable chromosome spreads	No. of chromosomes (In brackets - No. of chromatids) in MII							
		Haploid		Hyper-haploid			Hypo-haploid		
		20 (40)	240	22 (44)	21 (42)	20+1C* (41)	19+1C* (39)	19 (38)	18+1C* (37)
470	390	240	1 (0.25%)	30 (7.7%)	27 (6.9%)	50 (12.8%)	40 (10.9%)	1 (0.25%)	1 (0.25%)
		58 (14.9%)			92 (23.6%)				
TOTAL 390		Normal 240 (61.5%)			Abnormal 150 (38.5%)				

\*1C - One unpartnered chromatid



**Fig. 4.** Air-dried preparation from one-cell stage T6/+ mouse embryo, treated with okadic acid. Hyperhaploid set ( $n=21$ ) of G1-chromosomes derived from the 2nd PB. The marker chromosome  $15^{14}$  is clearly recognizable (arrow). C-banding with silver, counter-staining with Giemsa. ( $\times 980$ ).

the nucleus of the 2nd PB and from the MPN, a small T6 marker chromosome  $15^{14}$  was recognizable.

Therefore, it was possible to distinguish between the groups of G1-chromosomes derived from the 2nd PB and from the MPN and/or PPN. A cytogenetic analysis was performed on 903 one-cell stage embryos (Table 2.). In 265 cases (29.3%) only the nucleus of the 2nd PB changed in a presumably haploid group of separated and countable G1-chromosomes, while the pronuclei were presented by clawed single-chromatid chromosomes unified in a radial configuration. In 615 embryos (71.4%) the 2nd PB and both pronuclei were transformed in three presumably haploid groups of chromosomes which simulated metaphase plates and were suitable for a cytogenetic analysis. Three hundred and fifty seven embryos (58%) were euploid, i.e. contained 20 chromosomes in the MPN and in the 2nd PB, and in 258 embryos (48%) the aneuploid complements were found (Table 3).

The cytogenetic analysis revealed all predicted types of abnormality in chromosome sets of the 2nd PB and the MPN expected in the case of aneuploidy due to non-disjunction and to chromatid abnormal segregation. One hundred and eight embryos (17.6%) presented hyper- and hypohaploidy (6.7 and 10.9%, respectively) caused by non-disjunction at the first meiotic division (Fig. 3, route 2a,b). In 124 embryos (20%) the aneuploid set of chromosomes was found either in the 2nd PB or in the MPN, whereas, the second set was euploid and haploid. In these embryos aneuploidy derived from abnormal segregation of a chromatid at the first meiotic division (Fig. 3, route 3). In 26 embryos (4.4%) the total sum of chromosomes in the 2nd PB and the MPN equalled 40, being euploid and diploid, but the sets of chromosomes in the 2nd PB and in the corresponding MPN were complementary, i.e., the 2nd PB



**Fig. 5.** The same embryo. Hyperhaploid set ( $n=21$ ) of G1-chromosomes derived from the corresponding MPN. The chromosome  $15^{14}$  is marked with arrow. C-banding with silver, counter-staining with Giemsa. ( $\times 980$ ).

was hyperhaploid ( $n+1$ ) and the MPN hypohaploid ( $n-1$ ), or vice versa. In these cases aneuploidy originated at the second meiotic division (Fig. 3, route 4).

In Table 4 the results of cytogenetic analysis of the PPN and a group of 2nd PB's are presented. This group of 2nd PB's belonged

TABLE 2

**SUITABLE FOR CYTOGENETIC ANALYSIS G1-CHROMOSOMES IN T6/+ EMBRYOS AND/OR IN CORRESPONDING SECOND POLAR BODIES**

No. of examined embryos	No. of embryos with analyzable G1-chromosome spreaded						
	Total	Only in 2PB	In 2PB, MPN and PPN	In 2PB and in MPN	In 2PB and in PPN	In MPN	Only in MPN
1157	903 78%*	265 29,3%	288 31,9%	327 39,5%	11 1,2%	6 0,7%	6 0,7%
			615** 71,4%				

\* - Estimated from total No. of examined embryos

\*\* - This group was used for evaluation of chromosome constitution of the 2nd PB and the MPN (Tab. 3)



to the embryos in which both pronuclei were inappropriate for cytogenetic studies. In 181 (68.3%) of the 2nd PB's the euploid and haploid complement was detected, while in 37 (14%) cases hyperhaploidy, and in 47 (18.1%) hypohaploidy was registered.

Almost all (45 from 46) hypohaploid 2nd PB's contained 19 chromosomes, and most of the hyperhaploid 2nd PB (35 from 37) were with 21 chromosomes. Unlike in the 2nd PB only in a few PPN aneuploidy was found. In 350 PPN (92.3%) the euploid and haploid set of chromosomes was registered, 10 pronuclei (2.7%) were hyperhaploid with 21 chromosomes, and 12 (3.2%) were hypohaploid and contained 19 chromosomes. In 7 PPN (1.8%) the structural chromosome aberrations (gaps, breaks) were detected.

**Discussion**

The investigation was carried out on female mice heterozygous for reciprocal translocation T[14;15]6Ca. Then, and this is what should be taken into consideration, there are grounds for so-called tertiary non-disjunction which results from the abnormal segregation of chromosomes 14 and 15 participating in a complex meiotic configuration (quadrivalent) formed by the translocated (rearranged) chromosomes and their non-translocated homologs (for review see Searle, 1989). It was shown that in meiosis of T6/+ females the alternate disjunction yields two main types of euploid gametes with a balanced genotype, while adjacent disjunction gives four different types of euploid gametes with a non-balanced genotype and two types of aneuploid gametes (for review see Dyban and Baranov, 1987). The chromosome banding technique not being used in the present study, it was not possible to determine embryos with structural chromosomal abnormalities (duplications and deficiencies). In other words, only the number of oocytes and embryos with numerical chromosomal aberrations were counted.

A mention should be made that in the present work an unconventional cytogenetic approach was used. It consisted in counting prematurely condensed G1-chromosomes in the MPN and in the 2nd PB. This method, never applied before for evaluation of aneuploidy, urgently needs verification, and the assessment of the obtained data should be done with caution. As far as the metaphases of secondary oocytes are concerned, they were studied by a routine cytogenetic technique.

Now it would be logical to make a comparison of the data obtained in the study of MII-chromosomes in oocytes and the results of our analysis of G1-chromosome sets in the embryos and in the 2nd PB's. According to our observations, 14.9% of MII oocytes were hyperhaploid and 23.6% hypohaploid, i.e., 38.5% of T6/+ oocytes were aneuploid. If we add together the number of hyper- and hypohaploid T6/+ oocytes detected by Oshimura and Takagi (1975) and by Kaufman (1976), we will have the figures 36% and 37.8%, i.e. the results of these two studies performed on a small number of oocytes are in good agreement with our finding.

To summarize the number of hypo- and hyperhaploid oocytes one must be certain that hypohaploidy is not the result of artificial losses of chromosomes that occur because of over-spreading. It is apparent that over-spread metaphases can (and should) be excluded. We strictly complied with this principle and counted chromosomes only in the plates which were looking as complete. In our work the chromosomal preparations were made by the technique, according to which oocytes and fertilized embryos were spread after fixation. This prevented tearing of cell membrane and the



**Fig. 6. Air-dried preparation from one-cell stage T6/+ mouse embryo, treated with okadic acid. Hyperhaploid set (n=21) of G1-chromosomes derived from the 2nd PB. The marker chromosome 15<sup>14</sup> is clearly recognizable (arrow). C-banding with silver, counter-staining with Giemsa. (x 980).**

scattering of chromosomes was minimized. Furthermore, in one-cell stage embryos the spreading of chromosomes was performed when the remnants of the cytoplasm were still present. Due to this, the chromosomes were dispersed on the slide within a limited surface, i.e. the zone of cytoplasmic remnants, which made it possible to find a recoiled chromosome. Despite the attempts to prevent artifacts, the question may arise whether hypohaploidy was real or artificial.

A crucial argument which was made evident in the present study that the absence in MII of a chromosome is not a technical artifact, stems from comparison of the incidence of hypohaploidy in the oocytes and in the one-cell stage embryos. According to our data the rate of incidence of MII oocytes with 19 chromosomes (40 from 390) was the same as the incidence of embryos with 19 chromosomes in the 2nd PB and in the MPN (67 from 615, i.e. 10.9%).

Noteworthy is almost full conformability of the rate of hyperhaploidy due to the presence of one extra chromosome in the oocytes and in the fertilized embryos. Thus, 7.7% of MII oocytes (30 from 390) were with 21 chromosomes and 6.7% of embryos (41 from 615) had 21 chromosomes in each of the 2nd PB and the MPN. It should be mentioned that G1-chromosome sets in the PPN can serve as additional internal control of the efficiency of our cytogenetic analysis. In T6/+ embryos the PPN's were derived from spermatozoa of the CBA/C57BL mice with normal karyotype, which implies the low incidence of aneuploidy. The data in Table 4 fully supports this assumption and shows, that while 14% of the 2nd PB's were hyperhaploid, only in 2.6% of PPN hyperhaploidy was present. The same difference was observed in the rate of hypohaploidy (17.7% versus 3.2%). This speaks in favor that our technique correctly detects the aneuploidy in one-cell stage embryo.

Oshimura and Takagi (1975) reported that some of that T6/+ oocytes contained one unpaired chromatid, and our studies con-



**Fig. 7.** The same embryo. Haploid set ( $n=20$ ) of G1-chromosomes derived from the corresponding MPN. C-banding with silver, counterstaining with Giemsa. ( $\times 980$ ).

forms this observation. An unpartnered chromatid *per se* is not the abnormality which is typical only of T6/+ oocytes. MII oocytes with single unpartnered chromatids were occasionally found in different mouse strains and in hamsters (Polani and Jagiello, 1976; Hansmann and El-Nahass, 1979; de Boer and van der Hoeven, 1980; Mikamo and Kamiguchi, 1983; Hummler *et al.*, 1987). Unpaired chromatids have been previously reported in oocytes following chemical treatment (Hummler and Hansmann, 1985; Tease and Fisher, 1986; Mailhes *et al.*, 1990; Mailhes and Marchetti, 1994). The presence of single unpaired chromatid, in addition to (or replacing) a whole chromosome, was recently described in human MII oocytes (Angell *et al.*, 1993, 1994).

According to some authors, unpaired chromatid appeared as a result of premature centromere separation or early segregation of daughter chromatids at prometaphase II, and MII oocytes with only one single chromatid may have originated from technical artifacts in which one chromatid was lost during slide preparation (Mailhes *et al.*, 1990). The results of our observation are not consistent with such interpretation. Indeed, had the unpaired chromatid in MII

oocyte been artifact, then there would have been no aneuploidy that was predicted to form after abnormal segregation of chromatid in the first meiotic division (Fig. 3 routes 3 a,b,c,d). Furthermore, the incidence of unpaired chromatid in oocytes (20%) is the same as the rate of aneuploidy in the one-cell stage embryos formed by route 3. Thus, our data shows that unpartnered chromatid is not a technical artifact, and supports the «presegregation» («predivision») hypothesis, which postulated that chromatid abnormal segregation at the first meiotic division leads to this abnormality in the secondary oocytes (for review see Hansmann, 1983; Angell, 1991, Gosden and West, 1993; Angell *et al.*, 1994).

It should be accentuated that according to the literature data, unpaired chromatid in the mouse oocytes was detected only in 1-3% of the cases, while we found that 20% of T6/+ oocytes had this abnormality, and in all our cases unpaired chromatid was presented as a small marker chromosome 15<sup>14</sup>. This suggests the chromosome 15<sup>14</sup> to have increased predisposition to premature centromere division, which facilitates the formation of unpartnered chromatid during the first meiotic division.

The idea was put forward about the impact of unpartnered chromatid in oocytes in the subsequent embryonic aneuploidy in mouse (Sakurada *et al.*, 1994 and references therein) and in human (Angell, 1991, Angell *et al.*, 1993, 1994). To our knowledge we have obtained the first direct proof that secondary oocytes with unpaired chromatid are fertilized, followed by the appearance of 4 classes of embryos, including those which are to be monosomic and trisomic. Since the MPN of these embryos have 20, 21 or 19 chromosomes a standard metaphase analysis will classify some as euploid, and others as hyper- and hypohaploid due to non-disjunction of a whole chromosome at the first meiotic division. We found aneuploidy derived in the second meiotic division in 4.4% of cases. The MPN in some embryos were with 21 and in other with 19 chromosomes, and also could be mistakenly estimated as a result of non-disjunction of a whole chromosome at the first meiotic division.

It means that the analysis of G1-chromosome sets in the MPN and in the corresponding 2nd PB allows to distinguish the conse-

TABLE 3

CHROMOSOME CONSTITUTION OF 2PB AND MPN IN T6/+ EMBRYOS

Total No. of embryos*	No. of G1-chromosomes in 2PB and in corresponding MPN										
	20/20	21/21	19/19	21/20	20/21	20/19	19/20	21/19	19/21	18/22	22/18
615	357	41	67	33	36	24	31	9	11	4	2
	58.0%	6.7%	10.9%	5.3%	5.8%	3.9%	5.0%	1.5%	1.8%	0.7%	0.4%
TOTAL	357	108**		124***			26****				
	58.0%	17.6%		20.0%			4.4%				

\* - No. of one-cell stage embryos with analyzable G1- chromosomes spreads in the 2PB and in the MPN

\*\* - Numerical aberrations resulted from non-disjunction at the first meiotic division ( Fig. 3 , route 2 )

\*\*\* - The consequence of chromatid malsegregation at the first meiotic division ( Fig. 3 , route 3 )

\*\*\*\* - Numerical aberrations produced at the second meiotic division ( Fig. 3 , route 4 )



TABLE 4

**CHROMOSOME CONSTITUTION OF THE SECOND POLAR BODIES\* AND THE PATERNAL PRONUCLEI IN T6/+ EMBRYOS**

Group	No. of analyzed	With No. of G1-chromosomes					With structural chromosomal abnormalities
		20	22	21	19	18	
2nd PB	265	181	2	35	46	1	-
		68.3%	0.8%	13.2%	17.7%	0.4%	
PPN	379	350	-	10	12	-	7
		92.3%		2.6%	3.2%		1.8%

\* - This group of the 2nd PB was studied in cases when the MPN and the PPN were non-suitable for cytogenetic analysis

quences of non-disjunction and abnormal chromatid segregation in the first and in the second meiotic divisions and to estimate accurately the incidence of different types of maternal meiotic errors. Our results also support the suggestion made on the basis of a mathematical model (Kola *et al.*, 1993) that a total incidence of aneuploidy should be determined by adding together the number of hyper- and hypohaploid oocytes, including those with unpartnered chromatid.

According to our data in 24.4% of embryos the chromosome complement of the 2nd PB differed from the chromosome sets in the corresponding MPN. This discrepancy, which reflects the occurrence of chromatid abnormal segregation, made evident that attempts to predict the karyotype in T6/+ embryos by cytogenetic analysis of isolated 2nd PB would be unsuccessful due to high incidence of emerged errors. Although OA treatment (Dyban *et al.*, 1992) or fusion with zygote (Yerlinsky *et al.*, 1994; Evsikov and Evsikov, 1995) visualized in isolated mouse 2nd PB chromosomes suitable for cytogenetic study, the results of the present work contradict the idea that cytogenetic analysis of isolated 2nd PB can be used for selecting one-cell stage embryos with and without maternal numerical chromosomal aberrations. The false positive and the false negative errors in these studies are quite possible and the incidence of failures would correspond to the rate of chromatid abnormal segregation at the first meiotic division. Therefore the reliability of cytogenetic diagnosis of aneuploidy with the karyotype of isolated 2nd PB is questionable.

Noteworthy are gaps and breaks in the chromatid that were detected only in the PPN. Since the PPN were studied at the G1-phase of the cell cycle, it seems quite possible that some of these structural chromosomal aberrations could undergo reparation during the DNA synthesis. This unexpected finding, which needs further investigation by another technique, speaks in favor of the fact that the induction by OA of the prematurely condensed interphase chromosomes can be useful in the studies of DNA reparation in one-cell stage embryos.

**Materials and Methods**

**Mice**

Homozygous for reciprocal translocation T[14;15]6Ca (for review see Searle, 1989) CBA/CaH-T6/J males were obtained from the Jackson

Laboratory, and crossed with F1 CBA/C57BL females. In the study 10-12 week old females heterozygous for T[14;15]6Ca (onwards T6/+ mice) were used.

**Collection of oocytes and one-cell stage embryos**

The T6/+ mice were injected with 7.5 IU pregnant mares' serum gonadotropin (PMSG, Sigma), followed 46-48 h later by 7.5 IU human chorionic gonadotropin (hCG, Serono). Nineteen-20 h after the hCG injection the ovulated MII oocytes were removed from the ampullar region of the oviduct, treated with M-2 medium (Quinn *et al.*, 1982) containing 200 IU/ml hyaluronidase (Sigma) for removing cumulus cells, and then placed in a hypotonic solution for making the chromosomal preparations. For obtaining the one-cell stage embryos T6/+ females were caged (after the hCG injection) overnight with adult F1 CBA/C57BL males. Seventeen -18 h after the hCG injection the females with vaginal plugs were killed by cervical dislocation, the tubal eggs with cumulus masses were released in warm (+37°C) M-2 medium with hyaluronidase and fertilized eggs with clearly visible 2nd PB were selected to be treated with OA.

**Okadaic acid treatment**

A stock solution (1mM, i.e., 0.8 mg/ml) of OA (Sigma) in dimethyl sulphoxide (Sigma) was aliquotted and stored at -20°C. Immediately before the experiment, an aliquot of the stock solution of OA was thawed and added to M16-medium (Whittingham, 1971) in order to obtain the final concentration (5 µM) of OA. The fertilized eggs were transferred to drops of M-16 medium with OA and incubated for 45 min under the oil (at 37°C in 7.5% CO<sub>2</sub> -in-air). Then the eggs were carefully washed in 5-7 consecutive drops of plain M-16 medium, further cultured in this medium under the same conditions for 45 min, and then used for making chromosomal preparation.

**Chromosomal preparations**

Air-dried chromosome spreads from non-fertilized MII oocytes and from one-cell stage embryos treated with OA were made according to the method described earlier (Dyban, 1983, 1991) with the following modification. The non-fertilized MII oocytes were treated for 15-20 min at room temperature with a standard hypotonic solution (0.56% KCl), transferred to a watch glass with cold standard fixative (3:1 methanol and glacial acetic acid) and then were spread on a grease free thoroughly cleaned slide by the consecutive action of one drop of softening solution A (1:1 of 75% acetic acid and methanol) and one drop of softening solution B (1:1 methanol and glacial acetic acid) afterward the slide was dried out. The fertilized eggs were treated for 10 min with cold (4°C) slightly hypotonic solution (1.93% trisodium citrate). Then the egg was transferred to the fixative (4:1 methanol and glacial acetic acid), whereupon it was placed on slide and spread with a drop of softening solution B, taking special precaution to prevent the scattering and loss of chromosomes. Wherefore the outline of the egg was carefully observed under the stereomicroscope and when the egg was flattened, but before its cytoplasm fully disappeared, the slide was dried out by gentle blowing. Then 2-3 drops of standard fixative were put on the slide and it was again allowed to dry. All slides were stained according to the modified method of Howell and Black (1980) for visualizing the C-heterochromatin in chromosomes of oocytes and zygotes (Dyban *et al.*, 1990). Most of the slides were counter-stained for 1-2 min in Giemsa («Accuostain», Sigma, 2.5% in deionized water). The slides were examined and photographed using an Opton Microphot-3 microscope (oil immersion objectives 50 X and 100 X).

**Acknowledgments**

Part of the material presented in this paper was collected during the stay of A. Dyban at the Institute of Reproductive Genetics of the Masonic Medical Center in Chicago. The work was partly financially supported by the Russian Foundation for Basic Research (grant 94-04-48729) and the program "Priority Trends in Genetics" (Grant 2.247.). A.D. is grateful to the Institute of Reproductive Genetics for facilities and financial support which made possible the experiments on T6/+ embryos.



## References

- ANGELL, R. (1991). Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. *Hum. Genet.* 86: 383-387.
- ANGELL, R., XIAN, J. and KEITH, J. (1993). Chromosome anomalies in human oocytes in relation to age. *Hum. Reprod.* 8: 1047-1054.
- ANGELL, R., XIAN, J., LEDGER, W. and BAIRD, T. (1994). First meiotic division abnormalities in human oocytes: mechanism of trisomy formation. *Cytogenet. Cell Genet.* 65: 194-202.
- ANTONARAKIS, S.E., PETERSEN, M.B., MCINNES, M.G. and ADELSBERG P.A. (1992). The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphism. *Am. J. Hum. Genet.*, 50: 544-550.
- BOND, D.C. and CHANDLEY, A. (1983). *Aneuploidy*. Oxford Monograph on Medical Genetics No. 11, Oxford University Press, Oxford, pp 1-280.
- CHANDLEY, A. (1987). *Aneuploidy: An Overview in Aneuploidy Part A*, Incidence and Etiology (Eds. B.K.Vig, A.Sandberg), Alan Liss, New York, pp. 1-8.
- COHEN, P. (1989). The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* 58: 453-508.
- COHEN, P., HOLMES, C.F.B. and TSUTIKANI, Y. (1990). Okadaic acid: a new probe for the study of cellular regulation. *Trends Biochem. Sci.* 15: 98-102.
- DE BOER, P. and VAN DER HOEVEN, F. (1980). The use of translocation-derived marker bivalents for studying the origin of meiotic instability in female mice. *Cytogenet. Cell Genet.* 26: 49-58.
- DYBAN, A. (1983). An improved method for chromosomal preparations from preimplantation mammalian embryos, oocytes or isolated blastomers. *Stain Technol.* 58: 69-72.
- DYBAN, A. (1991). Reliable technique for chromosomal preparations from mammalian oocytes, preimplantation embryos and isolated blastomers. In *Preimplantation Genetic* (Eds. Y. Verlinsky, A. Kuliev), Wiley-Liss, New York, pp 293-298.
- DYBAN, A., and BARANOV, V. (1987). *Cytogenetics of Mammalian Embryonic Development*. Clarendon Press, Oxford, pp 1-340.
- DYBAN, A., DE SUTTER, P., DOZORTSEV, D. and YERLINSKY, Y. (1992). Visualization of second polar body chromosomes in fertilized and artificially activated mouse oocytes treated with okadaic acid. *J. Assist. Reprod. Genet.* 9: 572-579.
- DYBAN, A., DE SUTTER, P. and YERLINSKY, Y. (1993a). Okadaic acid induces premature chromosome condensation reflecting the cell cycle progression in one-cell stage mouse embryos. *Mol. Reprod. Dev.* 34: 402-415.
- DYBAN, A., DE SUTTER, P. and YERLINSKY, Y. (1993b). Preimplantation cytogenetic analysis. In *Preimplantation Diagnosis of Genetic Diseases* (Eds. Y. Verlinsky, A. Kuliev) Wiley-Liss, New York, pp. 93-127.
- DYBAN, A., SEVEROVA, E., ZATSEPINA, O. and CHENTSOV, Y. (1990). The silver stained NOR and argentophilic nuclear proteins in early mouse embryogenesis: a cytological study. *Cell Differ. Dev.* 29: 165-179.
- EVSIKOV, A.V. and EVSIKOV, S.V. (1995). A study of the first and second polar bodies in mouse oogenesis. *Ontogenez (Russ. J. Dev. Biol.)* 26:196-200.
- GAULDEN, M.E. (1992). Maternal age effect: the enigma of Down syndrome and other trisomic conditions. *Mutat. Res.* 296: 68-88.
- GOSDEN, J.R. and WEST, J.D. (1993). Prospects for preimplantation diagnosis of genetic diseases. In *Genes in Mammalian Reproduction* (Ed. R.B.L. Gwatkin), Wiley-Liss, New York, pp. 73-130.
- HANSMANN, I. (1983). Factors and mechanisms involved in non-dijunction and X-chromosome loss. In *Cytogenetics of the Mammalian X Chromosome. Part A. Basic Mechanisms of X Chromosome Behavior*. (Ed. A.A. Sandberg), Alan Liss, New York, pp. 131-170.
- HANSMANN, I. and EL-NAHASS, E. (1979). Incidence of non-disjunction in mouse oocytes. *Cytogenet. Cell Genet.* 24: 115-121.
- HASSOLD, T.J., PETTAY, D., FREEMAN, S.B., GRANTHAM, M. and TAKAESU, N. (1991). Molecular studies of non-disjunction in trisomy 16. *J. Med. Genet.* 28: 159-162.
- HOWELL, W.W. and BLACK, D.A., (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloid developer: A 1-step method. *Experientia* 36: 1014-1015.
- HUMMLER, E. and HANSMANN, I. (1985). Preferential non-disjunction of specific bivalents in oocytes from Djungarian hamsters (*Phodopus sungorus*) following colchicine treatment. *Cytogenet. Cell Genet.* 39: 161-167.
- HUMMLER, E., THEURING, F. and HANSMANN, I. (1987). Meiotic non-disjunction in oocytes from aged Djungarian hamster correlated with an alteration in meiosis rate but not in univalent formation. *Hum. Genet.* 4: 357-364.
- KAUFMAN, M.H. (1976). The incidence of chromosomally unbalanced gametes in T(14;15)6Ca heterozygote mice. *Genet. Res. Camb.* 27: 77-84.
- KOLA, I., SATHANATHAN, H. and GRASS, L. (1993). Chromosomal analysis of pre-implantation mammalian embryos. In *Handbook of In Vitro Fertilization* (Eds. A.Trounston, D. Gardner). CRC Press, Boca Raton, pp. 173-193.
- MAILHES, J.B. and MARCHETTY, F. (1994). Chemically-induced aneuploidy in mammalian oocytes. *Mutat. Res.* 320: 87-111.
- MAILHES, J.B., ZHI PING YUAN and AARDEMA, M.J. (1990). Cytogenetic analysis of mouse oocytes as a potential assay for heritable germ cell aneuploidy. *Mutat. Res.* 242: 89-100.
- MCLAREN, A. (1985). Prenatal diagnosis before implantation: opportunities and problems. *Prenat. Diagn.* 5: 85-90.
- MIKAMO, K. and KAMIGUCHI, Y. (1983). Primary incidences of spontaneous chromosomal anomalies and their origins and causal mechanism in the Chinese hamster. *Mutat. Res.* 108: 265-278.
- MODLINSKI, J. and MCLAREN, A. (1980). A method for visualizing the chromosomes of the second polar body of the mouse egg. *J. Embryol. Exp. Morphol.* 60: 93-97.
- OSHIMURA, M. and TAKAGI, N. (1975). Meiotic disjunction in T(14;15)6Ca heterozygotes and fate of chromosomally unbalanced gametes in embryonic development. *Cytogenet. Cell Genet.* 15: 1-16.
- PACCHIEROTTI, F. (1988). Chemically induced aneuploidy in germ cells of mouse. In *Aneuploidy Part B. Induction and Test Systems* (Eds. B.K.Vig, A.Sandberg), Alan Liss, New York, pp. 123-139.
- POLANI, P. and JAGELLO, G. (1976). Chiasmata, meiotic univalents and age in relation to aneuploid imbalance in mice. *Cytogenet. Cell Genet.* 16: 505-529.
- QUINN, P., BARROS C. and WHITTINGHAM D.S. (1982). Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa. *J.Reprod. Fertil.* 66: 161-168.
- SAKURADA, K., OMOE, K. and ENDO, A. (1994). Increased incidence of unpartnered chromatids in metaphase II oocytes in 39,X(XO) mice. *Experientia* 50: 502-505.
- SCHONTHAL, A. (1992). Okadaic acid-a valuable new tool for the study of signal transduction and cell cycle regulation? *New Biologist.* 4: 16-21.
- SEARLE, A. (1989). Chromosomal variants. Numerical variants and structural rearrangements. In *Genetic Variants and Strains of the Laboratory Mouse*. (Eds. M. F. Lyon, A.Searle). Oxford University Press, Oxford, pp. 582-598.
- TEASE, C. (1988). Radiation-induced aneuploidy in germ cells of female mammals. In *Aneuploidy Part B, Induction and Test Systems* (Eds. B.K.Vig, A.Sandberg), Alan Liss, New York, pp. 141-157.
- TEASE, C. and FISHER, G. (1986). Oocytes from young and old female mice respond differently to colchicine. *Mutat. Res.* 173: 31-34.
- WHITTINGHAM, D.G. (1971). Culture of mouse ova. *J. Reprod.Fertil. (Suppl.)* 14: 7-21.
- YERLINSKY, Y., DOZORTSEV, D. and EVSIKOV S. (1994). Visualization and cytogenetic analysis of second polar body chromosomes following its fusion with one-cell stage mouse embryo. *J. Assist. Reprod. Genet.* 11: 123-131.