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# Mechanisms and consequences of paternally transmitted chromosomal abnormalities

F. Marchetti, A. J. Wyrobek

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# MECHANISMS AND CONSEQUENCES OF PATERNALLY TRANSMITTED CHROMOSOMAL ABNORMALITIES

Francesco Marchetti and Andrew J. Wyrobek

Biosciences Directorate

Lawrence Livermore National Laboratory, Livermore, CA 94550, USA

Send correspondence to:

Dr. Francesco Marchetti

Biosciences Directorate, L-448

Lawrence Livermore National Laboratory, Livermore CA 94550

Tel: (925) 423 6853

Fax: (925) 424 3130

e-mail: marchetti2@llnl.gov

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**ABSTRACT**

Paternally transmitted chromosomal damage has been associated with pregnancy loss, developmental and morphological defects, infant mortality, infertility, and genetic diseases in the offspring including cancer. There is epidemiological evidence linking paternal exposure to occupational or environmental agents with an increased risk of abnormal reproductive outcomes. There is also a large body of literature on germ cell mutagenesis in rodents showing that treatment of male germ cells with mutagens has dramatic consequences on reproduction producing effects such as those observed in human epidemiological studies. However, we know very little about the etiology, transmission and early embryonic consequences of paternally-derived chromosomal abnormalities. The available evidence suggests that: 1) there are distinct patterns of germ cell-stage differences in the sensitivity of induction of transmissible genetic damage with male postmeiotic cells being the most sensitive; 2) cytogenetic abnormalities at first metaphase after fertilization are critical intermediates between paternal exposure and abnormal reproductive outcomes; and, 3) there are maternally susceptibility factors that may have profound effects on the amount of sperm DNA damage that is converted into chromosomal aberrations in the zygote and directly affect the risk for abnormal reproductive outcomes.

## INTRODUCTION

Chromosomal abnormalities transmitted through male and female gametes are associated with pregnancy loss, developmental and morphological defects, infant mortality, infertility, and genetic diseases in the offspring including cancer (Hassold et al., 1996; McFadden and Friedman, 1997; Wyrobek et al., 2000; Hassold and Hunt, 2001). Constitutive chromosomal abnormalities occur in an estimated 20-50% of all human conceptuses, 30-50% of these are aborted before recognized pregnancy, and about 20% of all recognized pregnancies are lost before term (Wilcox et al., 1988; Tommerup, 1993). About 0.6% of liveborn infants have chromosomal abnormalities (Shelby et al., 1993). More than 80% of these abnormalities are *de novo* events that originate in the parental germ cells. Constitutive trisomies (e.g., trisomy 21, 18, 13) have large maternal contribution (Hassold et al., 1996), while the male contribution is more substantial for sex chromosomal aneuploidies (Hassold, 1998; Hassold and Hunt, 2001). For most chromosomes, aneuploid conceptuses are generally not viable with death occurring in windows of development that depend on the specific chromosome involved in the trisomy. Only those with sex chromosomal aneuploidy or one of these autosomal trisomies (chromosome 13, 18 and 21) survive to birth. Like sex chromosome aneuploidies, *de novo* germinal point mutations and structural rearrangements also arise mainly during spermatogenesis rather than oogenesis (Olson and Magenis, 1988; Chandley, 1991; Crow, 2001).

Despite the health risks to the developing embryo and offspring, little is known about the etiology of paternally-derived chromosomal abnormalities, however, male age is thought to be a contributing factor (Sloter et al., 2004). There is epidemiological evidence linking paternal exposure to occupational or environmental agents with an increased risk of spontaneous abortions and other problems in their offspring, including birth defects and cancer (Narod et al.,

1988; Olshan et al., 1991; Savitz et al., 1994; Olshan, 1995). Lifestyle of moderate cigarette smoking and alcohol consumption has been associated with increases in aneuploid sperm (Robbins et al., 1997; Rubes et al., 1998). There is also a large body of literature on paternally transmitted chromosomal aberrations in rodents showing 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 lethality, heritable translocations, malformations and cancer in the offspring (Kirk and Lyon, 1984; Shelby, 1996; Nomura et al., 2004). These rodent findings are the cornerstone of our understanding of the link between specific exposures, mechanisms of transmission, and an increased frequency of birth defects.

During the past decade, fluorescence *in situ* hybridization (FISH) has evolved into an efficient approach for detecting chromosomally abnormal sperm in humans (Wyrobek et al., 1990; Holmes and Martin, 1993), domestic animals (Rubes et al., 1999) and rodents (Lowe et al., 1996; Lowe et al., 1998). Since its introduction, human sperm FISH technology has shifted from using any chromosome for which a DNA probe was available to chromosomes with clinical relevance in human aneuploidy syndromes (i.e., 21, 18, 13, X and Y) (Frias et al., 2003). Another advance has been the development of assays to detect sperm carrying chromosomal structural aberrations such as terminal duplications, deletions and chromosomal breaks (Van Hummelen et al., 1996; Sloter et al., 2000). Sperm FISH technology and its application to detect the induction of chromosomal abnormalities in sperm of humans and rodents will not be addressed here (see recent reviews by Wyrobek et al., 2005a, 2005b). Here we will focus on methods for detecting paternally transmitted chromosomal defects in the zygote and embryos, the mechanisms involved in the induction and transmission of chromosomal damage and the consequences of transmitted damage on proper embryonic development.

## UNIQUENESS OF SPERM AND ZYGOTE BIOLOGY AFFECTS PATERNALLY TRANSMITTED DAMAGE

The amount and type of damage that are transmitted by the sperm at fertilization are a direct consequence of the special biology of the spermatogenesis and the zygotic stage of mammalian embryogenesis. Specifically, the latter part of spermatogenesis is DNA repair deficient (Figure 1) and genetic lesions induced during this period may accumulate in sperm and persist until fertilization; (b) zygotes are unique among cells because all cellular functions between fertilization and transcriptional activation of the embryonic genome rely on stored maternal products (Figure 2), which suggest that any repair of sperm DNA lesions depends on the maternal genome. Both these features are briefly described in the following sections.

### Mammalian spermatogenesis

Spermatogenesis is a complex and highly regulated differentiating system that can be divided in three phases: 1) the proliferative phase is initiated from stem cells through numerous sequential divisions of spermatogonia to form spermatocytes (meiotic cells); 2) the meiotic phase in which spermatocytes go through recombination and two meiotic divisions to give rise to haploid spermatids (postmeiotic cells); and, 3) the spermiogenesis phase during which spermatids undergo major morphological and biochemical changes to form mature spermatozoa. Spermatogenesis is regulated by strict controls of the expression of genes encoding proteins that play essential roles during specific periods of germ cell development (Hecht, 1998; Grootegoed et al., 2000; Kleene, 2001). Both transcriptional and translational control mechanisms are

responsible for temporal and stage-specific expression patterns (Kleene, 1996; Braun, 1998; Eddy and O'Brien, 1998; Sassone-Corsi, 2002). Equally important is the establishment and the maintenance of epigenetic modifications that are required for the proper expression of paternally-derived genes during normal embryonic development (Trasler, 1998; Kerjean et al., 2000; Reik et al., 2001; Reik and Walter, 2001).

The kinetics of spermatogenesis are well established for men and several mammalian species and are remarkably constant within species (Oakberg, 1956; Adler, 1996). As shown in Figure 1, it takes approximately 35 days in mice (~64 days in humans) for germ cells to develop from spermatogonia to spermatozoa. The last round of DNA synthesis occurs in preleptotene spermatocytes. Meiotic prophase lasts about two weeks in the mouse (less than 4 weeks in man) and is followed by the first and second meiotic divisions, which occur within 24 hr of each other, while spermiogenesis takes about three weeks (over 5 weeks in man). Spermatids become repair deficient during late postmeiosis (Sega, 1979; Sotomayor and Sega, 2000) when the nucleus undergoes major chromatin restructuring (Meistrich, 1989; Wouters-Tyrou et al., 1998) and epigenetic reprogramming (Hazzouri et al., 2000). The somatic and meiotic histones that remain in spermatids are replaced ~14 days before ejaculation in the mouse (~21 days in human) with basic transition proteins and then with protamines (Meistrich, 1989), which are arginine-rich proteins that condense the chromatin and cause DNA to become transcriptionally inactive (Kierszenbaum and Tres, 1978). Sperm are then released into the seminiferous lumen and undergo a final process of maturation within the epididymis where they acquire motility and the ability to fertilize the egg (Gatti et al., 2004).

As discussed later, the special biology of spermatogenesis has important relevance to the types of DNA lesions and chromosomal defects that can be carried by the sperm to the egg.

**Zygotic cell cycle**

The first embryonic cell cycle has G1, S, G2, and M phases (Figure 2), but there are dramatic deviations from normal somatic cells that may have important implications for the fate of paternally transmitted damage: (1) the fertilizing sperm nucleus undergoes major chromatin alteration during G1 as it decondenses and the protamines are removed and replaced by histones (Garagna and Redi, 1988; Nonchev and Tsanev, 1990; Perreault, 1992); (2) the male and female pronuclei proceed to mitosis as distinctly separate pronuclei (McGaughey and Chang, 1969); (3) The genomes of both pronuclei are generally thought of being inactive until they form a single nucleus at the beginning of the two-cell stage in the mouse (Nothias et al., 1995; Latham and Schultz, 2001; Schultz, 2002). The male pronucleus seems to be transcriptionally more permissive than the female pronucleus during the one cell stage suggesting that the chromatin of paternal and maternal pronuclei may function differently (Majumder and DePamphilis, 1995; Nothias et al., 1995; Schultz and Worrad, 1995; Latham and Schultz, 2001; Schultz, 2002). In mice, the first evidence of endogenous transcription is detected late during G2 (Bouniol et al., 1995) as the zygote approaches the first mitotic division (Figure 2), however, the full activation of the embryonic genome, or zygotic gene activation (ZGA), does not occur until the 2-cell stage in the mouse (Schultz and Worrad, 1995; Schultz, 2002; Hamatani et al., 2004; Zeng et al., 2004), and even later in the human embryo (Braude et al., 1988). Thus, the majority of cellular events that takes place during the first few cell cycles of development occurs in the absence of transcription under the control of mRNAs and proteins stored in the egg before fertilization. As we will discuss later, this has important implications for the fate of sperm DNA damage transmitted to the embryo.

**Genetic defects that can be transmitted by the sperm**

The risk for paternally transmitted genetic defects is influenced by a number of factors, such as abnormal male reproductive physiology, predisposing genetic factors (Hassold and Hunt, 2001), past and present environmental exposures or random errors during sperm production (Crow, 2001). Table 1 lists the types of genetic defects that may be transmitted via sperm. Whole- and segmental chromosomal aneuploidies can result in complete or partial trisomy in offspring, respectively (Hassold and Hunt, 2001). DNA breaks and premutational lesions can arise spontaneously, and can be induced by exposure to mutagenic agents. Trinucleotide repeat length variation appear to be inducible in male germ cells after exposures to ionizing radiation (Dubrova et al., 1996; Dubrova et al., 2000; Barber et al., 2002) or environmental pollution (Somers et al., 2002; Somers et al., 2004). Sperm carrying defects in the imprinting profiles may alter the expression of paternal genes during critical stages of development resulting in abnormal development or defects (Doerksen and Trasler, 1996).

The likelihood that each of these defects is induced depends on the specific biology of the germ cell stage. For example, aneuploidy originates by segregation errors during the two meiotic divisions, while postmeiosis is the most vulnerable phase of spermatogenesis for the induction of DNA lesions that can be transmitted by the sperm. Treatment of male mice with germinal mutagens during the postmeiotic window of spermatogenesis results in a variety of sperm lesions including strand breaks, DNA adducts, and protamine adducts (Sega et al., 1989; Sega, 1991). Protamine adducts via the sulfur of cysteine are a category of damage unique to male germ-cells and is the predominant adduct generated by alkylating agents (Sega, 1991). Some produce both DNA and protamine adducts, while others, i.e., acrylamide, produce only protamine adducts with

no evidence of DNA adducts (Sega, 1991). With the diminished DNA repair during late postmeiosis, such sperm lesions may accumulate and be transmitted to the egg where they have the potential of being converted into chromosomal aberrations if improperly repaired. Indeed, Sega et al. (1989) showed a strong correlation between the time-course of adducts in mature sperm and the time-course of induction of embryonic lethality suggesting that sperm adducts are important intermediates between paternal exposure and abnormal reproductive outcomes.

## METHODS FOR INVESTIGATING PATERNALLY TRANSMITTED CHROMOSOMAL ABNORMALITIES

A variety of methods are available for the evaluation of genetic damage in male germ cells using conventional or FISH staining to measure endpoints such as numerical and structural aberrations, sister chromatic exchanges and micronuclei (Russo, 2000). However, these methods do not permit the assessment of the transmissibility of the defects to the offspring, which requires the analysis in the progeny. In the next few sections, we will describe the most relevant methods that have been used to investigate transmitted damage from the male to the progeny, focusing on those assays that detect chromosomal defects.

### Rodent breeding methods

Two of the most common breeding methods used for investigating paternally transmitted genetic defects have been the dominant lethal (DL) and the heritable translocation (HT) tests (Shelby et al., 1993). The DL test measures postimplantation embryonic death of the progeny of treated males presumably due to chromosomal abnormalities, while the HT test measures

chromosomal rearrangements in the offspring of treated males. These tests can be used to determine the sensitivity of the various spermatogenic cell types to the induction of genetic abnormalities by controlling the time between male exposure and mating. Matings within the first 3 weeks after exposure measure mutagenic effects on postmeiotic germ cells, while matings occurring 3-5 weeks, 5-7 weeks and more than 7 weeks after exposure measure effects on spermatocytes, spermatogonia and stem cells, respectively. About 30 chemicals have been tested for the induction of transmissible genetic defects in the mouse (Shelby, 1996). Figure 3 summarizes the results for those mutagens that have been tested in both methods and a few others for which DL data only is available but that have been tested for the induction of chromosomal aberrations using the cytogenetic analysis of zygotes. The majority of these mutagens are used as chemotherapeutic agents (Witt and Bishop, 1996; Wyrobek et al., 2005a), while others are used in various industrial processes and are also components of tobacco smoke (i.e., acrylamide, 1,3-butadiene and its metabolite diepoxybutane).

The results of these breeding tests show that there are distinct patterns of germ cell-stage differences in the sensitivity of induction of transmissible genetic damage. With the exception of etoposide and 6-mercaptopurine, all mutagens produced the highest response, if not exclusively, in male postmeiotic cells. Only, X-rays affected all stages of spermatogenesis, including spermatogonia. To date, no mutagen has induced transmissible chromosomal defects in stem cell spermatogonia and very few have induced transmissible gene mutations in these cells (Shelby, 1996). As mentioned earlier, the high sensitivity of postmeiotic cells is probably related to the reduced DNA repair capacity of late spermatids and sperm when compared with early spermatids and the other spermatogenic cell types (Sega, 1979; Sotomayor and Sega, 2000).

Another common finding was that, with the exception is 6-mercaptopurine and benzo[a]pyrene, positive results in the DL test were associated with positive results in the HT test. However there are quantitative differences. For the majority of the mutagens, there is a close relationship between the rates at which DL and HT mutations are induced, while others, (1,3-butadiene, chlormethine, ethyl nitrosourea, etoposide, isopropyl methanesulphonate, mitomycin C and procarbazine) have ratios greater than 10 to 1, i.e., are strong inducers of DL but only weak inducers of heritable translocations. The reasons for these differing responses are not fully understood. It has been suggested that the relative rates at which DL and HT mutations are induced depend on the types of chromosomal lesions present at the time the sperm fertilizes the egg. Only those mutagens whose premutational lesions are converted into breaks prior to DNA synthesis in the fertilized egg would produce similar rates of DL and HT mutations (Generoso et al., 1979b; Generoso, 1982). For etoposide, there is evidence that the high DL/HT ratio is due to a preferential induction of chromosomal fragmentation rather than exchanges (Marchetti et al., 2001). It is also possible that those mutagens with a high DL/HT ratio are inducing DL by mechanisms other that chromosomal abnormalities.

Mutagens that induce high rates of heritable translocations are of a greater concern than those that induce mostly dominant lethality, because heritable translocations represent a type of damage that is known to contribute to the human disease burden (Stenson et al., 2003; Abeysinghe et al., 2004). Reciprocal translocations have also provided crucial tools for the localization of genes associated with a variety of human cancers and hereditary diseases (Stubbs et al., 1997).

**Cytogenetic analysis of sperm chromosomes at zygotic metaphase**

The metaphase plate of the first mitotic division after fertilization provides the first opportunity for analyzing the chromosomal constitutions of the sperm after fertilization using conventional methods. Two of the most common methods developed for detecting paternally transmitted chromosomal aberrations are the human-sperm/hamster-egg cytogenetic method (or hamster-egg method) and the cytogenetic analysis of mouse zygotes. Both these tests have provided important information to help identify factors that may influence paternally transmitted chromosomal abnormalities and are discussed briefly below.

*The human-sperm/hamster egg method*

Originally described by Rudak et al. (Rudak et al., 1978), the hamster-egg method allows human sperm chromosomes to be examined at the first metaphase after fertilization in the hamster egg using standard cytogenetic staining techniques (Brandriff et al., 1985; Kamiguchi and Mikamo, 1986; Martin et al., 1986; Templado et al., 1988; Genesca et al., 1990b; Martin and Rademaker, 1990; Estop et al., 1991). It is based on the fusion of capacitated human sperm with hamster oocytes whose zona pellucidae were removed enzymatically. The hamster-egg method has been used to analyze sperm chromosome complements from healthy donors, men with constitutional chromosomal abnormalities (numerical and structural) and from cancer patients. 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 (reviewed in Guttenbach et al., 1997). Significant variation was also found in the frequencies of chromosomally abnormal sperm among healthy men, and these inter-donor

differences were reproducible over time (Brandriff et al., 1985), a finding recently confirmed using sperm FISH (Rubes et al., 2002). Moreover, men exposed to certain genotoxic agents (i.e., chemotherapy and radiation) exhibited higher frequencies of sperm with chromosomal aberrations compared to controls (Martin et al., 1986; Genesca et al., 1990a; Robbins, 1996; Martin et al., 1999).

The hamster egg method has played a critical role in the validation of sperm FISH assays (Wyrobek et al., 2000) and it is still considered the gold standard for the validation of new methods for detecting chromosomal defects in sperm. Although, highly informative, the hamster-egg method has been difficult and expensive to perform, only few laboratories around the world have mastered it, and the number of papers that has been published in the recent years has decreased significantly.

*Cytogenetic analysis of mouse zygote metaphases*

The cytogenetic analysis of mouse first-cleavage (1-Cl) zygotes was developed to investigate the induction and transmission of aneuploidy after exposure of female germ cells (Mailhes and Marchetti, 1994; Mailhes and Marchetti, 2005) and of structural aberrations after exposure of male germ cells (summarized in Table 2). The assay allows the identification of the parental origin of the induced abnormality because paternal and maternal chromosomes do not join until the metaphase stage of the first mitotic division (McGaughey and Chang, 1969) and because maternal chromosomes show a higher degree of condensation with respect to the paternal chromosomes (Donahue, 1972). Until a few years ago, the analysis of zygotic metaphases relied on standard cytogenetic techniques, such as Giemsa staining and C-banding, which are very effective at detecting unstable aberrations but ineffective at detecting stable

chromosomal rearrangements. However, the development of chromosome composite probes for the mouse (Breneman et al., 1993; Boei et al., 1994; Breneman et al., 1995) has opened the possibility of using chromosome painting for the study of heritable chromosome aberrations. The PAINT/DAPI method (Figure 4) combines DAPI staining to detect unstable aberrations such as dicentrics and acentric fragments, with chromosome-specific FISH painting probes to detect stable aberrations such as translocations and insertions (Marchetti et al., 1996; Marchetti and Wyrobek, 2003). As summarized below, the 1-Cl assay has been used in mouse studies of the effects of paternal exposure to mutagens to investigate: 1) the transmission of numerical abnormalities from the father to the offspring; 2) the induction of chromosomal aberrations in 1-Cl zygotes after paternal treatment with germ cell mutagens; and 3) the correlation between chromosomal aberrations at first cleavage and subsequent embryonic development.

*Paternal transmission of aneuploidy*

The investigation of the transmission of aneuploidy via sperm has taken advantage of the availability of strains of mice with reciprocal or Robertsonian translocations (Gropp et al., 1975; Oshimura and Takagi, 1975; Beechey and Searle, 1991). These lines of mice are known to produce high levels of aneuploid sperm because of segregation errors during meiosis and have been used to determine whether there is selection against aneuploid sperm at fertilization. Similar frequencies of aneuploid spermatocytes and preimplantation embryos (Ford, 1972) and of monosomic and trisomic preimplantation embryos (Epstein, 1985) provided indirect evidence that aneuploid sperm were equally effective at fertilization. Using Roberstonian translocation carriers we compared the frequencies of aneuploid sperm before and after fertilization (Marchetti et al., 1999). The results showed that there was very good agreement between the frequencies of

aneuploid male complements in metaphase II spermatocytes and zygotes. In particular, hyperhaploidy for chromosome 16 occurred in 20.0% of spermatocytes and in 21.8% of zygotes. Hypohaploidy for chromosome 16 occurred in 17.0% and 16.7% of spermatocytes and zygotes, respectively. These findings indicated that aneuploidy for chromosome 16 did not affect the fertilizing capacity of sperm. However, it remains possible that aneuploidy for other chromosomes and other types of chromosomal abnormalities may affect sperm maturation and fertilizing capacity. Indeed, results obtained with translocation carriers in Chinese hamsters suggested that sperm carrying specific chromosomal imbalances failed to participate in fertilization (Sonta et al., 1991; Sonta, 2004).

Several mutagens have been shown to increase the frequencies of anueploid sperm in rodents, mostly by chemicals that damage microtubules (reviewed in Mailhes and Marchetti, 2005; Wyrobek et al., 2005b). However, few chemicals have been studied for the transmission of chemically induced aneuploidy in male germ cells. Also, because it takes about 21 days for cells that were undergoing the two meiotic divisions at the time of treatment to reach the ejaculate, the majority of the studies, such as those shown in Table 2, analyzed sperm complements at time points that did not allow for the possible induction of aneuploidy to be investigated. Etoposide, a topoisomerase II inhibitor, is the only mutagen that has been shown to induce a significant increase in the frequencies of aneuploid zygotes of paternal origin (Marchetti et al., 2001).

*Paternal transmission of chromosomal structural aberrations*

The 1-Cl zygote studies that have been conducted to investigate the transmission of chromosomal structural aberrations after paternal exposure are summarized in Table 2. The majority of these studies have used standard cytogenetic staining techniques, and therefore, the

information obtained is limited to the induction of unstable aberrations. Information on the induction of stable as well as unstable aberrations is limited to the few studies that utilized the PAINT/DAPI technology (Marchetti et al., 1997; Marchetti et al., 2001; Marchetti et al., 2004).

Several dose-response and time-course studies are available for the transmission of chromosomal aberrations from male germ cells to the zygote. Dose-response studies utilizing three of more doses of the mutagen are available for acrylamide (Pacchierotti et al., 1994), 1,3-butadiene (Pacchierotti et al., 1998); diepoxybutane (Adler et al., 1995); EMS (Matsuda and Tobari, 1988); MMS (Brewen et al., 1975; Tanaka et al., 1981; Matsuda and Tobari, 1988); UV (Matsuda and Tobari, 1988) and X-rays (Matsuda et al., 1985; Matsuda et al., 1989c). With the exception of diepoxybutane, these studies generally reported a linear or nearly linear increase in the frequencies of chromosomal aberrations providing further evidence for the diminished repair of sperm lesions in postmeiotic germ cells. The time-response studies (Albanese, 1987; Matsuda et al., 1989c; Matsuda et al., 1989d; Marchetti et al., 2001; Marchetti et al., 2004) show patterns of sensitivity for the induction of paternally transmitted chromosomal aberrations that overlap the pattern of sensitivity for dominant lethality: namely, the highest frequencies of chromosomal aberrations are found after exposure of postmeiotic germ cells and very few mutagens induced significant increases after treatment of meiotic germ cells or spermatogonia.

A common finding of these studies is that regardless of the mutagen used or its mechanism of action, the majority of chromosomal aberrations detected at 1-Cl metaphase were of the chromosome-type, i.e. affecting both sister chromatids. Chromatid-type aberrations were very rare, even when S-phase dependent chemicals were used. Although it remains unclear why this is the case, it proves that double strand breaks are an obligatory intermediate step in the process and that these breaks occur during the G1 phase of the zygotic cell cycle. For alkylating

agents, it was suggested that the primary type of damage is protamine adducts that were induced in postmeiotic cells (Sega et al., 1989). The adducted protamines would create physical stresses in the chromatin structure leading to DNA double strand breaks that would result in chromosome-type aberrations. Alternatively, adducted protamines may be refractory to removal from DNA during pronuclear formation, and thus may indirectly function as "bulky DNA adducts". Other types of lesions, such as single strand breaks, base damages and apurinic or apyramidinic sites would be converted into double strand breaks by misrepair or enzymatic attack on the opposite strand during the G1 stage (Matsuda et al., 1989c). Only those rare lesions that would survive the G1 phase unchanged, would have the potential of originating chromatid-type aberrations during S-phase.

Overall these 1-Cl studies have shown that: 1) the majority of chromosomal aberrations induced after paternal exposure to mutagens are represented by chrosomome-type aberrations such as dicentrics and acentric fragments; 2) the highest amount of chromosomal damage is found after exposure of postmeiotic germ cells in agreement with the findings of the traditional breeding studies; 3) at high doses nearly all zygotes have paternally derived chromosomal aberrations suggesting that the presence of high amounts of DNA lesions does not affect the sperm fertilizing capacity; 4) the studies utilizing PAINT/DAPI analysis showed that different mutagens have different proportion of stable and unstable aberrations which may explain the differential results obtained with the DL and HT tests.

**Embryonic fate of paternally transmitted chromosomal aberrations**

The suggestion that chromosome structural aberrations were the most likely cause of the embryonic lethality observed in the DL tests was put forward long ago (Brewen et al., 1975).

Recently, we conducted a detailed investigation of the correlation between chromosomal aberrations at first cleavage and dominant lethality using the PAINT/DAPI method to simultaneously analyze zygotes for the presence of stable and unstable aberrations (Marchetti et al., 1996; Marchetti et al., 1997; Marchetti et al., 2001; Marchetti et al., 2004). These studies have shown that zygotic chromosomal abnormalities are quantitative intermediates between paternal exposure and abnormal reproductive outcomes. Specifically, 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, while the proportion of zygotes with stable aberrations was comparable with the frequencies of offspring with heritable translocations reported using the standard HT method (Marchetti et al., 2004).

The close correlation between unstable chromosomal aberrations in zygotes and embryonic lethality is further confirmed by the results shown in Figure 5, where the frequencies of zygotes with chromosomal aberrations as reported in Table 2 are compared with the frequencies of dead implants as reported in DL tests. When the comparison is limited to studies that utilized identical doses in both tests (9 mutagens, 41 datapoints, Figure 5A), the frequencies of zygotes with unstable aberrations are related to the DL frequencies in a linear one-to-one relationship that explained 81% of the variance between these endpoints (ß=0.73, $R^2$=0.81, P<0.001). Even when the comparison is extended to include those studies in which the doses used in the two testes differed by up to 25% (12 mutagens, 66 data points, Figure 5B), the correlation was still highly significant (ß=0.65, $R^2$=0.74, P<0.01). These results prove that chromosomal aberrations are the main cause of embryonic lethality following mutagen treatment of male germ cells.

As discussed by Marchetti et al. (2004), an implication of the close agreement between chromosomal aberrations in zygotes and postimplantaion embryonic lethality is the conclusion that embryonic development proceeds to implantation regardless of the presence of unstable chromosomal aberrations. The lack of stringent cell cycle checkpoints (Handyside and Delhanty, 1997) and of the apoptotic response (Brison and Schultz, 1997; Hardy et al., 2001) during these initial stages of embryonic development and the fact that this period of development is supported in large part by nutrients and factors stored in the egg before fertilization may allow embryos with unstable chromosomal aberrations to survive until implantation. It can be further speculated that embryos that have high levels of unstable chromosomal aberrations would result in early postimplantation death, while those embryos with few unstable chromosomal aberrations may be able to proceed further into development before dying (Marchetti et al., 2004).

The results of these comparisons unequivocally show that chromosomal structural aberrations are critical intermediates between paternal exposure and abnormal reproductive outcomes. They also show that the fate of the embryo is set by the end of the first cell cycle of development.

## ZYGOTIC REPAIR MECHANISMS FOR SPERM DNA LESIONS

The close correlation between chromosomal aberrations in zygotes and embryonic fate highlights the importance of the events that take place immediately after fertilization. The G1 phase of the first cell cycle of development plays a crucial role for normal development by repairing DNA damage in the paternal genome. However, very little is known about the mechanisms by which mutagen-induced sperm lesions are repaired in the zygote. Brandriff and Pedersen (1981) demonstrated that UV damage in sperm was processed in zygotes by measuring

radionucleotide grain counts over the male pronuclei. Evidence from a number of in vivo and in vitro systems indicates that the mammalian oocyte is capable of repairing a variety of DNA damage (Ashwood and Edwards, 1996) and transcripts for DNA damage response and repair genes are more abundant in the egg that in later preimplantation stages (Zeng et al., 2004). This suggests that the oocyte has been under evolutionary pressure to store DNA repair gene transcripts and proteins to assure the genomic integrity of the fertilizing sperm and of the egg DNA.

Several lines of evidence show the importance of DNA repair during the early stages of mammalian development. In the late 80's, Matsuda and colleagues performed a series of experiments with DNA repair inhibitors during the first cycle of development in mouse zygotes and showed that interference with DNA repair markedly altered the amounts and types of chromosomal abnormalities detected at 1-Cl metaphase after paternal exposure to X-rays and chemical agents (Matsuda et al., 1989a; Matsuda et al., 1989b; Matsuda and Tobari, 1989; Matsuda and Tobari, 1995). These studies suggested that several types of DNA lesions can be repaired in the zygote and provided compelling evidence that chromosomal aberrations were formed after fertilization rather than before it. Also, because both chromosome- and chromatid-type aberrations were affected, both pre- and post-replication repair mechanisms must be operating in the zygote. Similar findings were obtained using the hamster egg method (Genesca et al., 1992).

It is also known that female mice from different genotype/stocks can vary substantially in the fraction of DL observed after mating with males exposed to the identical dose of germ-cell mutagen (Generoso et al., 1979a; Bishop et al., 1983). The largest differential response for DL (~9-fold) was reported after isopropylmethanesulphonate (iPMS)-exposed males were mated

with (C3HxC57BL)F1 versus T-stock female mice. Of particular relevance, there was an associated 13-fold differential effect in chromosomal abnormalities at 1-Cl metaphase (Generoso et al., 1979a). These studies provide compelling evidence that mouse stocks can differ in their ability to convert sperm lesions into chromosomal damage in the zygote, and provide a strong argument that maternal genetics plays a major role in determining the efficiency of converting sperm lesions into chromosomal aberrations after fertilization and, thus, embryonic fate.

## CONCLUSIONS AND FUTURE DIRECTIONS

We have made significant progress in understanding the factors that may predispose male germ cells to the induction of genetic damage that can be transmitted to the next generation, the role of the fertilizing egg in modulating the amount of transmitted damage and the consequences of proper embryonic development. Our model (Figure 6) suggests that genetic damage in sperm is a consequence of the sensitivity of the various phases of spermatogenesis, exposure to environmental mutagens or random error during sperm production and can be divided in two major classes: the first class includes those alterations that are already fixed in the genome, such as aneuploidy, structural rearrangements and mutations, that are transmitted as such to the embryo. The second class includes those types of lesions, such as DNA adducts, protamine adducts, etc., in sperm that have the potential of being converted into chromosomal aberrations in the egg. Furthermore, our model suggests that maternal deficiencies in DNA damage and repair mechanisms may significantly alter the amount of these lesions that are converted into paternally transmitted chromosomal damage at first cleavage. Once the lesions are fixed into

chromosomal aberrations the fate of the embryo is set down a path that will lead to altered gene expression, death during pregnancy or birth with morphological and genetic defects.

Future efforts are needed to understand the mechanism of the germ-cell stage sensitivity for the various types of genetic defects. There is evidence that the nature of the mutations is dependent upon the germ cell stage and its repair capacity rather than upon the mutagen (Russell, 1994). Differential expression of DNA repair-related genes among various spermatogenic cell types has been reported for the rat, and it has been suggested that such differential expression contributes to the selective susceptibilities of germ cells to stress (Aguilar-Mahecha et al., 2001).

A better understanding is also needed of the molecular pathways by which zygotes process sperm DNA lesions after fertilization. The availability of transgenic mice with gene mutations related to specific DNA metabolism and repair genes (Friedberg and Meira, 2003) provide valuable tools for investigating how sperm DNA lesions are recognized by the egg and how the DNA repair capacity of the fertilized egg affects the amount of DNA damage that is converted into chromosomal aberrations in the zygote and the risk for abnormal reproductive outcomes. It has been recently demonstrated that p53-dependent cell cycle checkpoints and pronuclear cross talk between parental pronuclei are activated by DNA damage carried by the sperm (Shimura et al., 2002). There is also evidence suggesting that the introduction of DNA damage by irradiated sperm triggers genomic instability that can induce mutations in the unirradiated maternal genome (Niwa and Kominami, 2001).

Recent technological advances in mRNA amplification allow the use of the very small starting material that can be obtained from eggs and early embryos to perform genome wide expression studies and are beginning to reveal the complexity of gene expression during early stages of embryonic development (Ko et al., 2000; Hamatani et al., 2004; Wang et al., 2004;

Zeng et al., 2004). It was recently shown that fertilization after paternal exposure to cyclophosphamide altered transcription of specific genes in rat zygotes and early embryos (Harrouk et al., 2000a; Harrouk et al., 2000b). Clearly, alterations in the embryonic gene expression pattern may be another mechanism by which paternal exposure cause abnormal reproductive outcomes. It will be interesting to determine whether the effects depend on the spectra of genetic lesions in the fertilizing sperm and whether such transcriptional changes are occurring even in the absence of paternally transmitted genetic lesions. Also, the possibility that paternal exposure to mutagens may alter the epigenetic reprogramming during spermatogenesis and the early stages of mammalian development warrant further study.

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Table 1 – DNA and chromosomal defects that may be transmitted by sperm

| | |
|---|---|
| • **Aneuploidy** | • **Premutational lesions** |
| - sex chromosomes | - DNA adducts |
| - autosomes | - protamine adducts |
| | - single and double strand breaks |
| • **Structural aberrations** | |
| - duplications/deletions | |
| - rearrangements | • **Changes in the number of trinucleotide repeats** |
| • **Epigenetic changes** | |
| - imprinting | • **Gene mutations** |

Table 2 – Summaries of mouse studies of paternally transmitted chromosome structural aberrations evaluated at zygotic metaphase

| Mutagen | Dose[a] | Days post treatment | Germ cell analyzed | Zygotes with aberrations affected/Total | (%)[b] | Reference |
|---|---|---|---|---|---|---|
| Acrylamide | 0 | - | | 1/120 | 0.8 | Pacchierotti et al 1994 |
| | 75 | 7 | Sperm | 11/144 | 7.6 | |
| | 125 | 7 | Sperm | 31/118 | 23.6 | |
| | 50 x 5 | 7 | Sperm | 47/55 | 85.4 | |
| Acrylamide | 0 | - | | 2/300 | 0.7 | Marchetti et al 1996 |
| | 50 x 5 | 2-9 | Sperm-late spermatids | 161/271 | 59.4 | |
| Acrylamide | 0 | - | | 1/284 | 0.4 | Marchetti et al 1997 |
| | 50 x 5 | 3 | Sperm | 61/100 | 61.0 | |
| | 50 x 5 | 7 | Sperm | 95/125 | 76.0 | |
| | 50 x 5 | 9 | Elongated spermatids | 109/216 | 50.5 | |
| | 50 x 5 | 13 | Elongating spermatids | 26/164 | 15.9 | |
| | 50 x 5 | 21 | Round spermatids | 10/118 | 8.5 | |
| | 50 x 5 | 28 | Pachytene spermatocytes | 6/126 | 4.8 | |
| Busulfan | 0 | - | | 0/92 | 0 | Albanase 1987 |
| | 50 | 3 | Sperm | 10/76 | 13.1 | |
| | 50 | 8 | Elongated spermatids | 12/86 | 13.9 | |
| 1,3-Butadiene | 0 | - | | 5/341 | 1.5 | Pacchierotti et al 1998 |
| | 500 ppm | 1-7 | Sperm | 13/185 | 7.0 | |
| | 1300 ppm | 1-7 | Sperm | 17/117 | 14.5 | |
| | 1300 ppm | 8-14 | Spermatids | 8/127 | 6.3 | |
| Cyclophosphamide | 0 | - | | 2/298 | 0.7 | Albanese 1987 |
| | 100 | 3 | Sperm | 9/87 | 10.3 | |
| | 100 | 8 | Elongated spermatids | 36/116 | 31.0 | |
| | 100 | 15 | Round spermatid | 22/119 | 18.5 | |
| | 200 | 3 | Sperm | 11/37 | 45.9 | |
| | 200 | 8 | Elongated spermatids | 27/53 | 50.9 | |
| | 200 | 15 | Elongating spermatids | 22/64 | 32.8 | |
| | 200 | 22 | Diakinesis spermatocytes | 3/56 | 5.3 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 200 | 50 | Stem cell | 2/20 | 10.0 | |
| Cyclophosphamide | 0 | - | | 10/1469 | 0.7 | Marchetti et al 2004 |
| | 120 | 18 | Round spermatids | 54/179 | 30.2 | |
| Diepoxybutane | 0 | - | | 2/127 | 0.6 | Adler et al 1995 |
| | 17 | 7 | Sperm | 11/117 | 9.4 | |
| | 26 | 7 | Sperm | 17/115 | 14.8 | |
| | 34 | 7 | Sperm | 13/96 | 13.5 | |
| EMS | 0 | - | | 0/122 | 0 | Albanese 1987 |
| | 100 | 3 | Sperm | 2/117 | 1.7 | |
| | 100 | 8 | Elongated spermatids | 24/80 | 30.0 | |
| | 100 | 15 | Round spermatid | 6/93 | 6.4 | |
| EMS[c] | 0 | - | | 3/212 | 1.4 | Matsuda and Tobary 1988 |
| | 0.5 mg/ml | 0 | Sperm | 19/251 | 7.6 | |
| | 1 mg/ml | 0 | Sperm | 38/234 | 16.2 | |
| | 1.5 mg/ml | 0 | Sperm | 46/229 | 20.1 | |
| | 2 mg/ml | 0 | Sperm | 56/197 | 28.4 | |
| Etoposide | 0 | - | | 2/318 | 0.6 | Marchetti et al 2001 |
| | 80 | 25 | Pachytene spermatocytes | 42/254 | 16.8 | |
| | 80 | 35 | B spermatogonia | 11/146 | 7.5 | |
| 5-Fluorouracil | 0 | - | | 0/119 | | Albanese 1987 |
| | 250 | 15 | Round spermatids | 3/138 | 2.2 | |
| | 250 | 22 | Diakinesis spermatocytes | 4/124 | 3.2 | |
| | 250 | 29 | Pachytene spermatocytes | 9/145 | 6.2 | |
| iPMS | 0 | - | | 1/194 | 0.5 | Generoso et al 1979a |
| | 65 | 1-3 | Sperm | 61/168 | 36.2 | |
| Melphalan | 0 | - | | 10/1469 | 0.7 | Marchetti et al 2004 |
| | 7.5 | 23 | Pachytene spermatocytes | 124/226 | 54.9 | |
| Mitomycin C | 0 | - | | 0/205 | 0 | Albansese 1987 |
| | 5 | 3 | Sperm | 4/77 | 5.2 | |
| | 5 | 8 | Elongated spermatids | 20/110 | 18.2 | |
| | 5 | 15 | Round spermatids | 12/60 | 20.0 | |
| | 5 | 22 | Diakinesis spermatocytes | 2/71 | 2.8 | |
| | 5 | 29 | Pachytene spermatocytes | 4/51 | 7.8 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mitomycin C | 0 | - | | 5/345 | 1.4 | Matsuda et al 1989c |
| | 5 | 12 | Elongating spermatids | 17/185 | 9.2 | |
| | 5 | 16 | Round spermatids | 16/223 | 7.2 | |
| | 5 | 20 | Round spermatids | 9/139 | 6.5 | |
| MMS | 0 | - | | 1/213 | 0.5 | Albanese 1987 |
| | 50 | 3 | Sperm | 18/62 | 29.0 | |
| | 50 | 8 | Elongated spermatids | 49/60 | 81.7 | |
| | 50 | 15 | Round spermatids | 6/54 | 11.1 | |
| | 100 | 3 | Sperm | 66/117 | 56.4 | |
| | 100 | 8 | Elongated spermatids | 91/95 | 95.8 | |
| | 100 | 15 | Round spermatids | 20/96 | 20.8 | |
| MMS | 0 | - | | 0/193 | 0 | Matsuda et al 1989d |
| | 50 | 4 | Sperm | 105/211 | 49.8 | |
| | 50 | 8 | Elongated spermatids | 114/156 | 73.1 | |
| | 50 | 12 | Elongating spermatids | 150/232 | 64.7 | |
| | 50 | 16 | Round spermatids | 20/195 | 10.3 | |
| MMS | 0 | - | | 0/128 | 0 | Albanese 1982 |
| | 100 | 4 | Sperm | 27/49 | 55.1 | |
| | 100 | 8 | Elongated spermatids | 36/37 | 97.3 | |
| | 100 | 12 | Elongating spermatids | 28/45 | 62.2 | |
| | 100 | 16 | Round spermatids | 14/57 | 24.6 | |
| MMS | 0 | - | | 1/100 | 1.0 | Brewen et al 1975 |
| | 25 | 7 | Sperm | 6/50 | 12.0 | |
| | 25 | 8 | Elongated spermatids | 4/50 | 8.0 | |
| | 25 | 9-10 | Elongated spermatids | 9/89 | 10.1 | |
| | 50 | 4 | Sperm | 9/63 | 14.3 | |
| | 50 | 6 | Sperm | 23/50 | 46.0 | |
| | 50 | 10 | Elongating spermatids | 27/50 | 54.0 | |
| | 50 | 11 | Elongating spermatids | 21/50 | 42.0 | |
| | 50 | 12-13 | Elongating spermatids | 25/58 | 44.8 | |
| | 50 | 14 | Elongating spermatids | 9/50 | 18.0 | |
| | 50 | 16 | Round spermatids | 6/75 | 8.0 | |
| | 100 | 3 | Sperm | 26/50 | 56.0 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 100 | 6-7 | Sperm | 55/61 | 90.1 | |
| | 100 | 10 | Elongated spermatids | 50/50 | 100 | |
| | 100 | 12-13 | Elongating spermatids | 41/60 | 68.3 | |
| | 100 | 15 | Round spermatids | 5/45 | 10.0 | |
| | 100 | 16 | Round spermatids | 5/45 | 10.0 | |
| | 100 | 18 | Round spermatids | 5/50 | 10.0 | |
| MMS | 0 | - | | 10/1469 | 0.7 | Marchetti et al 2004 |
| | 40 | 7 | Sperm | 114/249 | 45.8 | |
| MMS[c] | 0 | - | | 3/212 | 1.4 | Matsuda and Tobari 1988 |
| | 25 $\mu$g/ml | 0 | Sperm | 17/319 | 5.3 | |
| | 50 $\mu$g/ml | 0 | Sperm | 52/334 | 15.6 | |
| | 75 $\mu$g/ml | 0 | Sperm | 84/240 | 35.0 | |
| | 100 $\mu$g/ml | 0 | Sperm | 164/294 | 55.8 | |
| MMS | 0 | - | | 0/78 | 0 | Tanaka et al 1981 |
| | 20 | 6-10 | Sperm-Elongated spermatids | 8/63 | 12.6 | |
| | 40 | 6-10 | Sperm-Elongated spermatids | 19/55 | 34.5 | |
| | 60 | 6-10 | Sperm-Elongated spermatids | 48/63 | 76.1 | |
| | 80 | 6-10 | Sperm-Elongated spermatids | 69/71 | 97.1 | |
| | 100 | 6-10 | Sperm-Elongated spermatids | 56/56 | 100 | |
| Trophosphamide | 0 | - | | 2/124 | 1.6 | Tiveron et al 1996 |
| | 100 | 7 | Sperm | 17/104 | 16.3 | |
| | 100 | 14 | Elongating spermatids | 27/88 | 30.4 | |
| | 150 | 7 | Sperm | 27/99 | 29.0 | |
| | 150 | 14 | Elongating spermatids | 61/111 | 54.9 | |
| | 150 | 21 | Round spermatids | 19/118 | 16.1 | |
| Trimethylmelanine | 0 | 0 | | 3/137 | 2.2 | Burki & Sheridan 1978 |
| | 0.2 | 1-7 | Sperm | 9/51 | 17.6 | |
| | 0.2 | 8-14 | Elongated spermatids | 12/63 | 19.0 | |
| | 0.2 | 15-21 | Round spermatids | 2/23 | 8.7 | |
| | 0.4 | 1-7 | Sperm | 18/52 | 34.6 | |
| | 0.4 | 8-14 | Elongated spermatids | 26/54 | 48.1 | |
| | 0.4 | 15-21 | Round spermatids | 4/31 | 12.9 | |
| Trimethylmelanine | 0 | - | | 0/99 | 0 | Albanese 1987 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 0.025 | 3 | Sperm | 5/44 | 11.4 | |
| | 0.025 | 8 | Elongated spermatids | 40/67 | 59.7 | |
| | 0.025 | 15 | Round spermatids | 19/69 | 27.5 | |
| UV[c] | 0 | - | | 5/285 | 1.8 | Matsuda & Tobari 1988 |
| | 1.9 J/m$^2$ | 0 | Sperm | 45/314 | 14.3 | |
| | 3.6 J/m$^2$ | 0 | Sperm | 69/305 | 22.6 | |
| | 5.4 J/m$^2$ | 0 | Sperm | 93/189 | 49.2 | |
| | 7.2 J/m$^2$ | 0 | Sperm | 65/95 | 68.4 | |
| X-rays | 0 | | | 5/345 | 1.4 | Matsuda et al 1989c |
| | 4 Gy | 0 | Sperm | 102/264 | 38.6 | |
| | 4 Gy | 4 | Sperm | 79/187 | 42.2 | |
| | 4 Gy | 8 | Elongated spermatids | 57/172 | 33.1 | |
| | 4 Gy | 12 | Elongating spermatids | 102/206 | 49.5 | |
| | 4 Gy | 16 | Round spermatids | 145/180 | 80.6 | |
| | 4 Gy | 20 | Round spermatids | 141/194 | 72.7 | |
| X-rays | 0 | - | | 10/1469 | 0.7 | Marchetti et al 2004 |
| | 4 Gy | 7 | Sperm | 53/624 | 20.1 | |
| X-rays | 0 | - | | 11/560 | 2.0 | Matsuda et al 1989a |
| | 5 Gy | 0 | Sperm | 109/261 | 41.8 | |
| X-rays[c] | 0 | | | 2/290 | 0.7 | Matsuda & Tobari 1989 |
| | 5 Gy | 0 | Sperm | 92/185 | 49.7 | |
| X-rays[c] | 0 | - | | 2/223 | 0.9 | Matsuda et al 1985 |
| | 0.5 Gy | 0 | Sperm | 10/225 | 4.4 | |
| | 1 Gy | 0 | Sperm | 21/230 | 9.1 | |
| | 2 Gy | 0 | Sperm | 36/231 | 15.6 | |
| | 3 Gy | 0 | Sperm | 70/261 | 26.8 | |
| | 4 Gy | 0 | Sperm | 95/247 | 38.5 | |
| X-rays[c] | 0 | - | 0 | 7/363 | 1.9 | Matsuda et al 1989b |
| | 1 Gy | 0 | Sperm | 48/341 | 14.1 | |
| | 2 Gy | 0 | Sperm | 79/287 | 27.5 | |
| | 3 Gy | 0 | Sperm | 106/274 | 38.7 | |
| | 4 Gy | 0 | Sperm | 142/272 | 52.2 | |
| | 5 Gy | 0 | Sperm | 138/215 | 64.2 | |

[a]Unless noted doses are in mg/kg.

[b]Only those experimental points with a significant increase in the frequencies of zygotes with structural aberrations are reported.

[c]Indicates studies where the exposure was conduted in vitro.

## FIGURE LEGENDS

**Figure 1**. Schematic of mammalian spermatogenesis with approximate duration of each phase in reference to the day of fertilization (Day 0) for men and mice. The approximate periods of DNA synthesis and DNA repair deficiency are indicated. Pl: preleptotene; L: leptotene; Z: zygotene; P: Pachytene; II: meiosis II.

**Figure 2.** Schematic of the first two cycles of mammalian development. Morphological and cell cycle events are indicated in relation to the of time of fertilization. See text for an explanation. Adapted from Majumder and De Pamphilis (1995).

**Figure 3.** Summary of studies of paternally induced dominant lethality and heritable translocatios. A scheme of mouse spermatogenesis with time between treatment and day of fertilization (day 0) is shown at the top. For each mutagen the mating intervals that produced significant increases with respect to control values are indicated. Color bars represent the relative frequencies of dominant lethality (below 25%, light bleu; between 26 and 50%, blue; above 50%, dark blue). Numbers in bars report the percentage of translocation carriers in the offspring. Gray boxes represent period of sterility induced after exposure. Original data was presented in the following papers (listed by mutagen): Acrylamide: (Shelby et al., 1986; Shelby et al., 1987; Adler et al., 1994a); Busulfan: (Ehling and Neuhauser-Klaus, 1991); Benzo[a]pyrene: (Generoso et al., 1982); 1,3-Butadiene: (Adler et al., 1998); Chlormbucil: (Generoso et al., 1995); Chlormethine: (Fox and Scott, 1980; Ehling and Neuhauser-Klaus, 1989); Cyclophosphamide: (Sotomayor and Cumming, 1975; Ehling and Neuhäuser-Klaus,

1988); Dacarbazine: (Adler et al., 2002); Diepoxybutane: (Adler et al., 1995); Ethylene oxide: (Generoso et al., 1986; Generoso et al., 1990); Ethyl methanesulphonate: (Cattanach et al., 1968; Ehling et al., 1968); Ethyl nitrosourea: (Generoso et al., 1984); Etoposide: (Bishop et al., 1997); Glycidamide: (Generoso et al., 1996); isopropyl methanesulfonate (Ehling and Neuhäuser-Klaus, 1995); (Generoso et al., 1979a); Melphalan: (Generoso et al., 1995); 6-mercaptopurine: (Generoso et al., 1975); Methyl methanesulfonate (Lang and Adler, 1977); Methyl nitrosourea: (Generoso et al., 1984); Mitomycin C: (Ehling, 1971; Adler, 1980); Procarbazine: (Ehling, 1974; Adler, 1980); Triethylenemelamine: (Cattanach, 1957; Bateman, 1960); Trophosphamide: (Adler et al., 1994b; Ehling and Neuhäuser-Klaus, 1994); X-rays: (Ehling, 1971; Searle et al., 1974).

**Figure 4.** PAINT/DAPI technology for the analysis of stable and unstable chromosomal abnormalities in mouse 1-Cl zygote metaphases. **A.** PAINT is used for detecting stable aberrations such as translocations and insertions. The most recent probe combination (Marchetti et al 2004) uses chromosome-specific painting probes for chromosomes 1, 2, 3, X and Y labeled with FITC and chromosomes 2, 4, 6 X and Y labeled with biotin and signaled with Texas Red™. **B.** DAPI staining is used for detecting unstable aberrations such as acentric fragments and dicentrics and any aberration not involving the painted chromosomes. **C.** Photomicrograph of a normal 1-Cl zygote metaphase with the X-bearing sperm-derived chromosomes on the left. Note that the paternal chromosomes show a lower degree of condensation with respect maternal chromosomes. **D**: 1-Cl zygote with X-bearing sperm-derived chromosomes showing a reciprocal translocation (arrows).

**Figure 5.** Linear regression analyses of the relationship between the frequencies of zygotes with unstable aberrations and subsequent dominant lethality. Panel A shows the comparion for those studies in which both tests used the same dose (Acrylamide, 1,3-Butadiene, Ethyl methanesulfonate, Etoposide, Melphalan, Methyl methanesulfonate, Trophosphamide, Triethylenemelamine and X-rays). Panel B includes those studies where the dose between the two tests differed by up to 25% (mutagens of Panel A plus Cyclophosphamide, Diepoxybutane, Mitomycin C).

**Figure 6.** Working model for the mechanisms of paternally transmitted chromosomal abnormalities and their consequences of proper embryonic development. See text for a description.

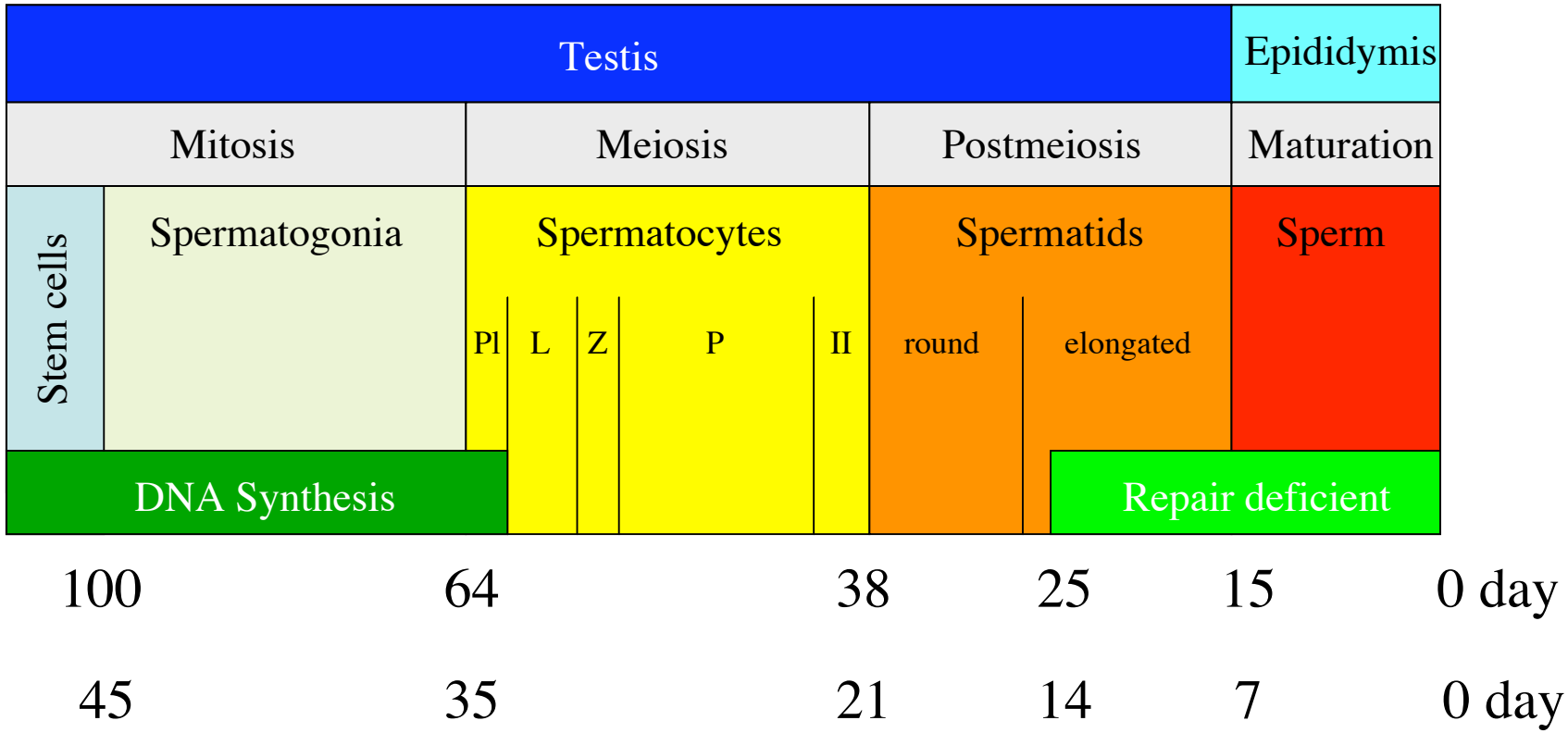
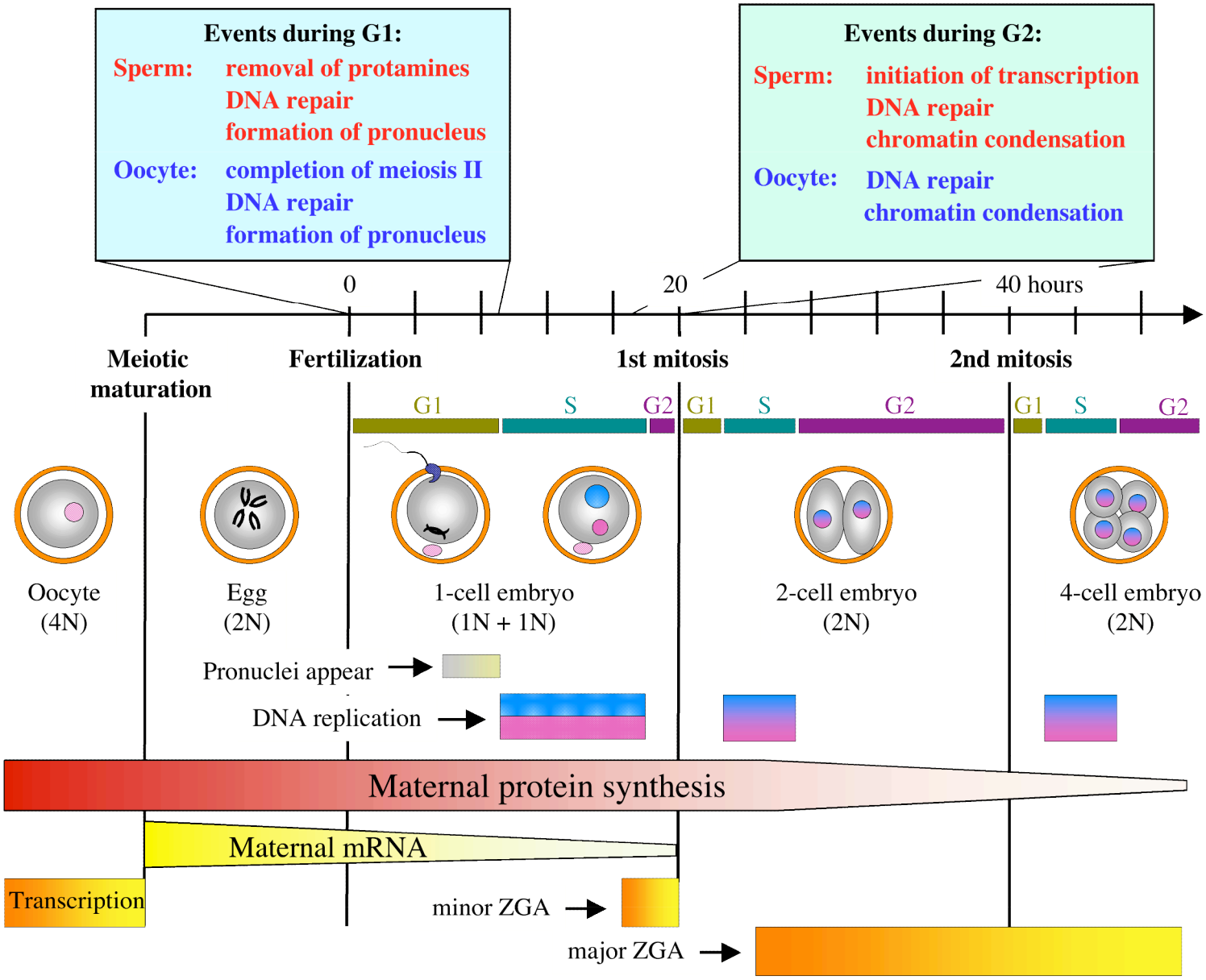

Testis
Epididymis
Mitosis
Meiosis
Postmeiosis
Maturation
Stem cells
Spermatogonia
Spermatocytes
Spermatids
Sperm
Pl
L
Z
P
II
round
elongated
DNA Synthesis
Repair deficient
100
64
38
25
15
0 day
45
35
21
14
7
0 day

Figure 1


Events during G1:
Sperm: removal of protamines
DNA repair
formation of pronucleus
Oocyte: completion of meiosis II
DNA repair
formation of pronucleus
Events during G2:
Sperm: initiation of transcription
DNA repair
chromatin condensation
Oocyte: DNA repair
chromatin condensation
0
20
40 hours
Meiotic maturation
Fertilization
1st mitosis
2nd mitosis
G1
S
G2
Oocyte (4N)
Egg (2N)
1-cell embryo (1N + 1N)
2-cell embryo (2N)
4-cell embryo (2N)
Pronuclei appear
DNA replication
Maternal protein synthesis
Maternal mRNA
Transcription
minor ZGA
major ZGA

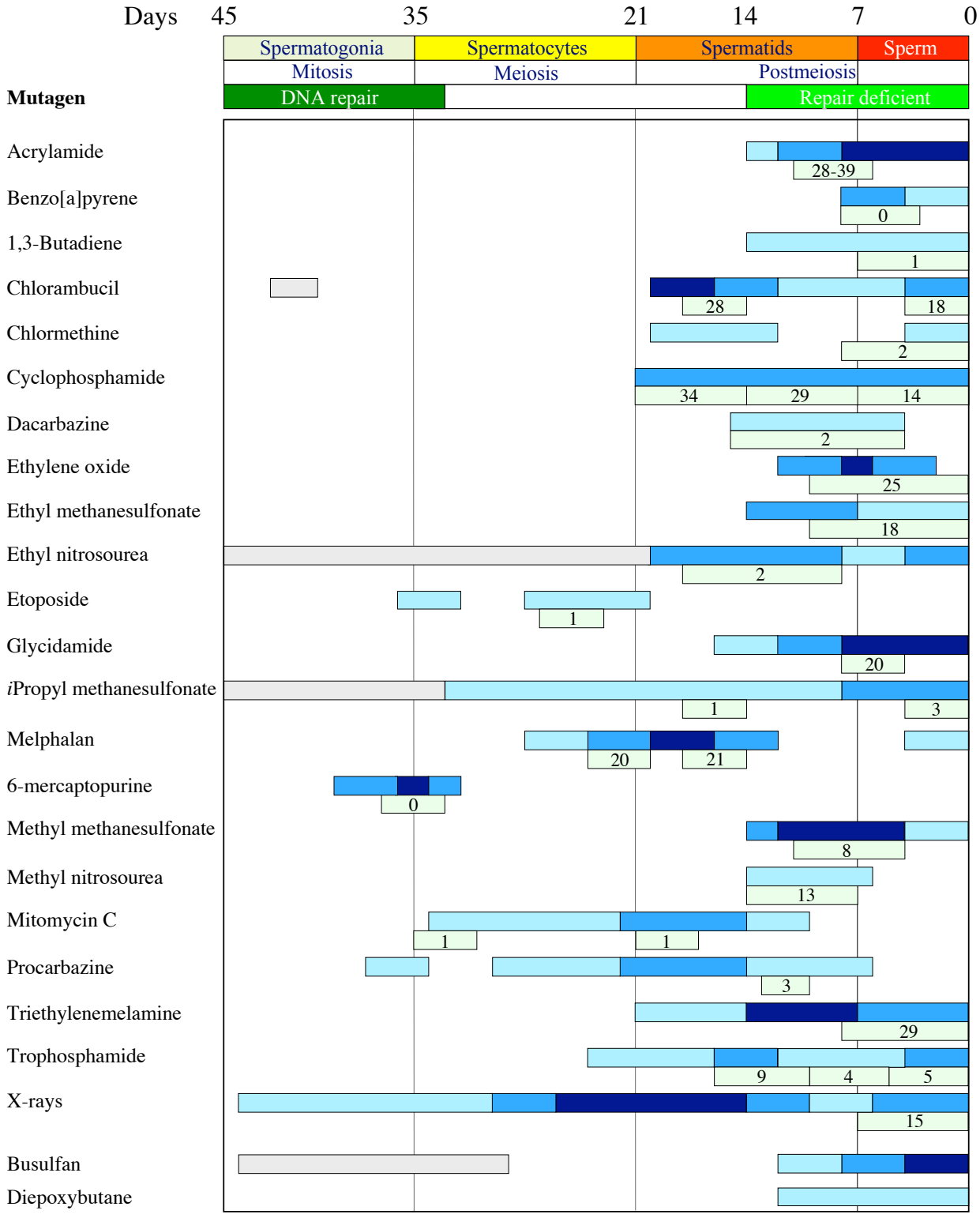

Days
45
35
21
14
7
0
Spermatogonia
Spermatocytes
Spermatids
Sperm
Mitosis
Meiosis
Postmeiosis
Mutagen
DNA repair
Repair deficient
Acrylamide
28-39
Benzo[a]pyrene
0
1,3-Butadiene
1
Chlorambucil
28
18
Chlormethine
2
Cyclophosphamide
34
29
14
Dacarbazine
2
Ethylene oxide
25
Ethyl methanesulfonate
18
Ethyl nitrosourea
2
Etoposide
1
Glycidamide
20
iPropyl methanesulfonate
1
3
Melphalan
20
21
6-mercaptopurine
0
Methyl methanesulfonate
8
Methyl nitrosourea
13
Mitomycin C
1
1
Procarbazine
3
Triethylenemelamine
29
Trophosphamide
9
4
5
X-rays
15
Busulfan
Diepoxybutane

Figure 3

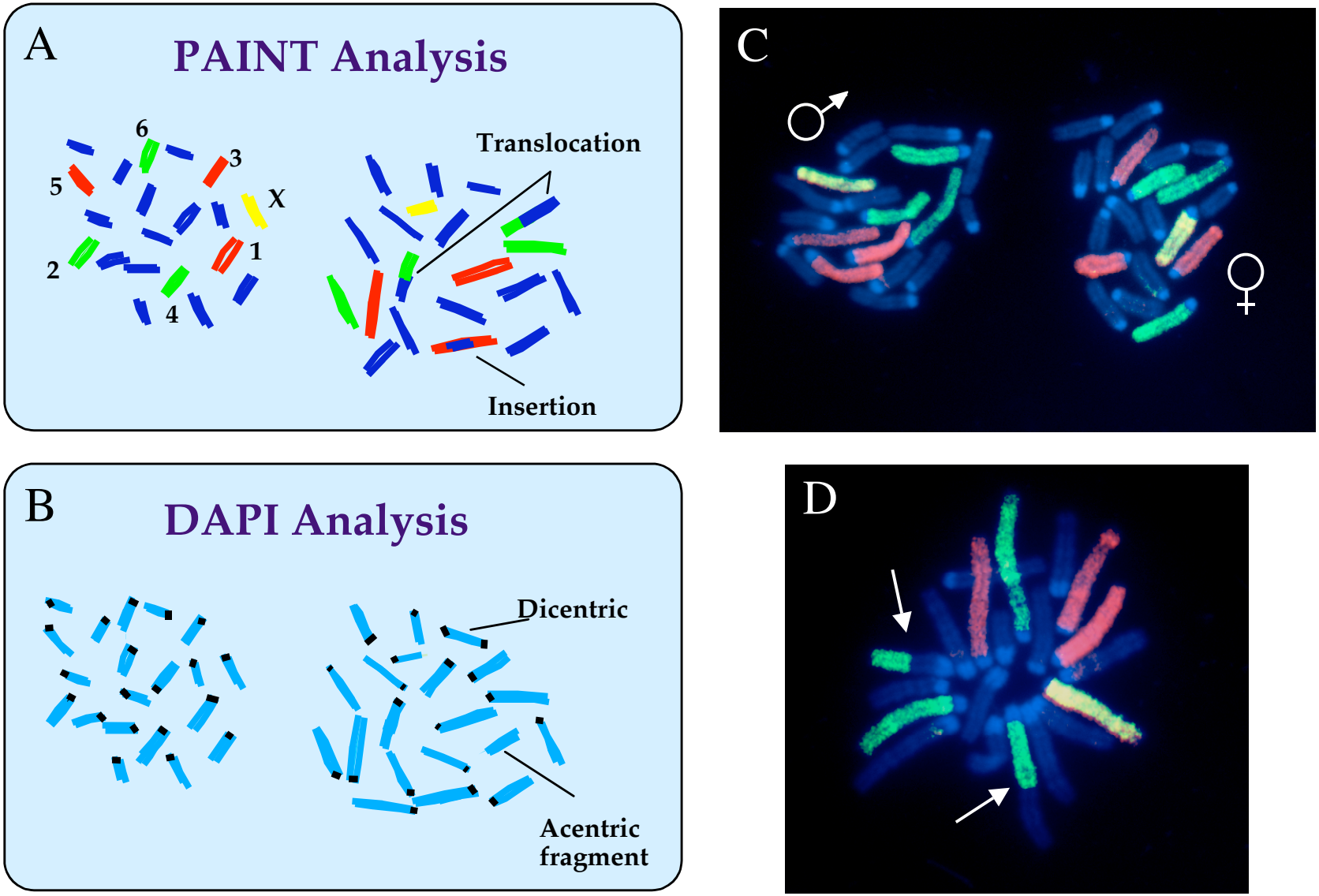
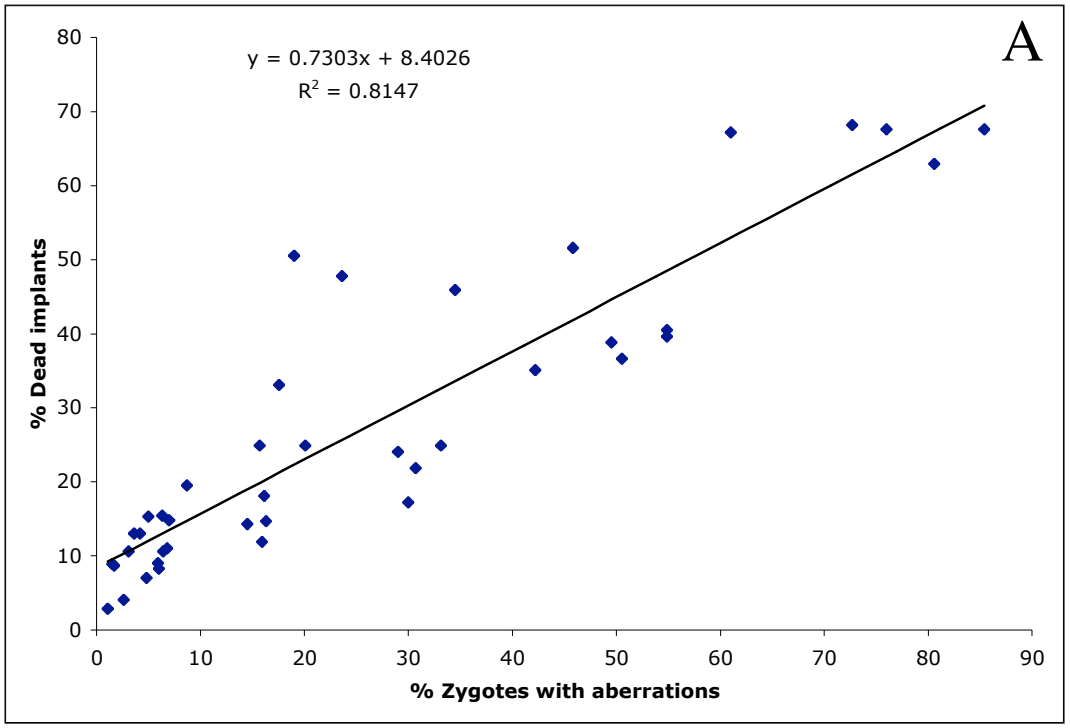

A
PAINT Analysis
6
3
5
X
1
2
4
Translocation
Insertion
B
DAPI Analysis
Dicentric
Acentric
fragment
C
♂
♀
D

Figure 4


A
y = 0.7303x + 8.4026
R2 = 0.8147
% Dead implants
% Zygotes with aberrations
0
10
20
30
40
50
60
70
80
90

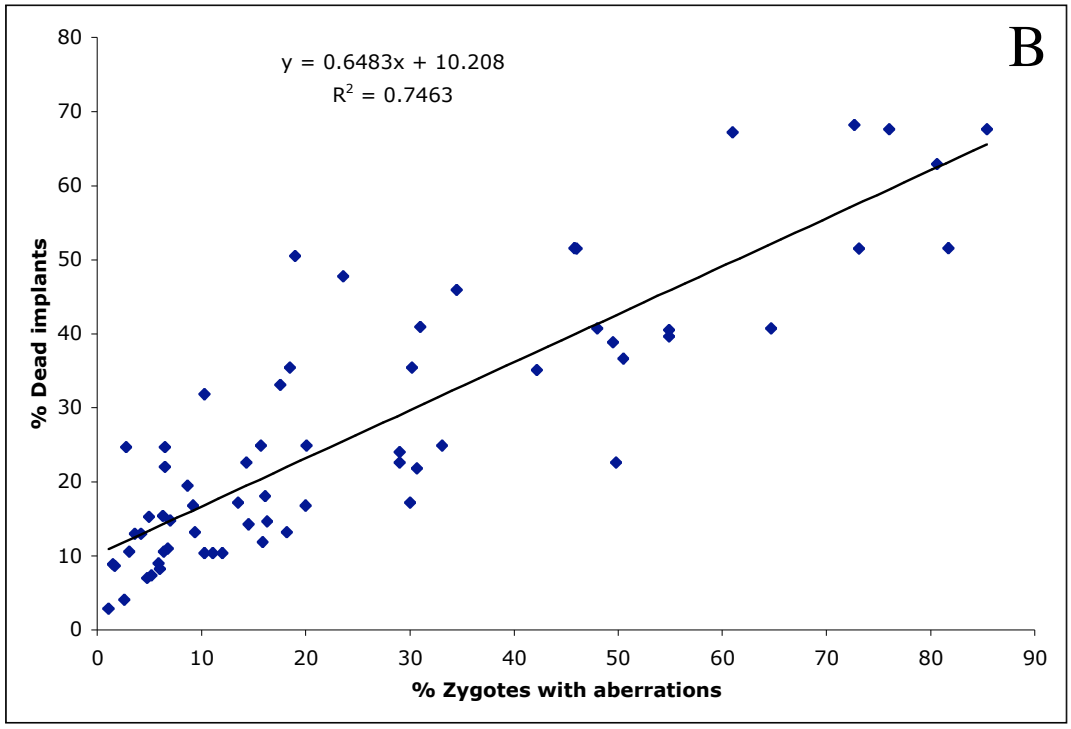

B
y = 0.6483x + 10.208
R2 = 0.7463
% Dead implants
% Zygotes with aberrations
0
10
20
30
40
50
60
70
80
90

Figure 5

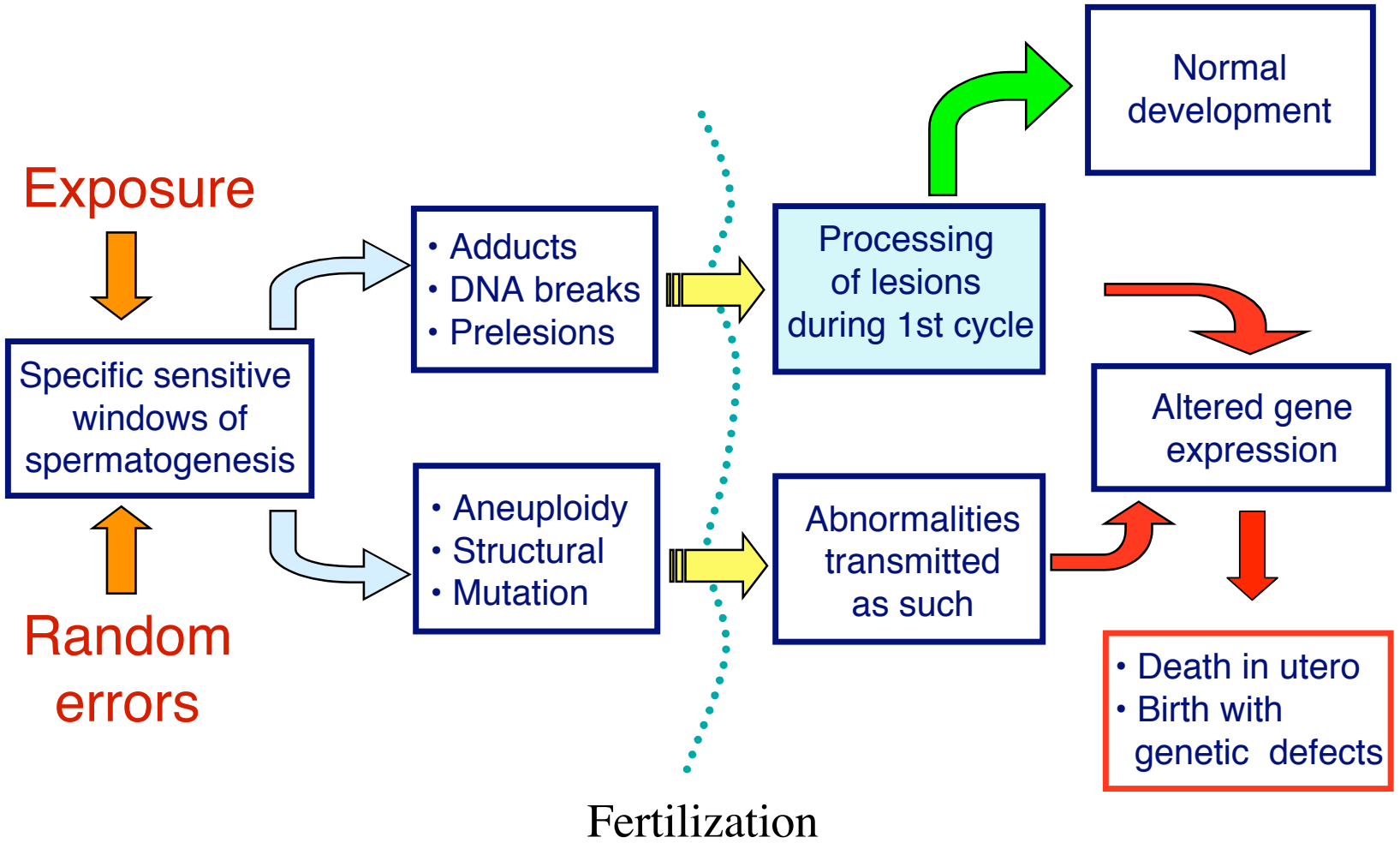

Exposure
Specific sensitive windows of spermatogenesis
Random errors
• Adducts
• DNA breaks
• Prelesions
• Aneuploidy
• Structural
• Mutation
Processing of lesions during 1st cycle
Abnormalities transmitted as such
Normal development
Altered gene expression
• Death in utero
• Birth with genetic defects
Fertilization

Figure 6