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Running head: Chromosomal analysis of mouse cleavage embryos

Key words: spermatozoa, γ-irradiation, early embryos, chromosomal aberrations, mouse

Abstract

***Purpose**:* To quantitatively and qualitatively investigate the changes in chromosomal aberrations during early cleavage in mouse embryos derived from γ-irradiated spermatozoa.

***Materials and methods**:* Mature males were exposed to 2 Gy or 4 Gy of ^{137}Cs γ-rays, and their spermatozoa were used to produce embryos via in vitro fertilization (IVF). The metaphase chromosomes were prepared from one-cell, two-cell, and four-cell embryos. In the chromosome preparations from two-cell and four-cell embryos, the separation of the sister blastomeres was precluded by treatment of the embryos with concanavalin A. The incidence of embryos with structural chromosomal aberrations, aneuploidy, or mosaicism was estimated. The fates of the different types of γ-ray-induced structural chromosomal aberrations were also investigated in those embryos.

***Results**:* The exposure of spermatozoa to 2 Gy or 4 Gy γ-rays caused structural chromosomal aberrations in 25.9% and 35.7% of the resultant one-cell embryos, respectively. At two-cell embryonic stage, the incidence of structural chromosome aberrations was 17.4% in the 2 Gy group and 27.1% in the 4 Gy group. At the four-cell embryonic stage, although the incidence of control embryos with structural chromosome aberrations was considerably high, the net incidence of embryos with radiation-induced structural chromosome aberrations was similar to that at the one-cell stage. The incidence of aneuploidy was high in two-cell and four-cell embryos after both doses of γ-rays. The incidence of mosaicism increased significantly in dose- and embryonic-stage-dependent manners. Anaphase lag, and the degeneration and nondisjunction of the aberrant chromosomes were frequently observed in aneuploid and mosaic embryos.

***Conclusions**:* Mouse sperm DNA is highly vulnerable to γ-rays. The structural

chromosomal aberrations of sperm origin are unstable in their behavior and structure during cleavage, and therefore cause secondary aneuploidy and mosaicism in the early cleavage embryos.

Introduction

Chromosomal analysis of one-cell embryos has been useful for measuring primary structural chromosomal damage in murine spermatozoa (Matsuda et al., 1985; 1989a,b; Matsuda and Tobari, 1989; Tateno et al., 1996a; Marchetti et al., 2004, 2007; Tusell et al., 2004; Kusakabe and Kamiguchi, 2004; Derijck et al., 2008) and human spermatozoa (Kamiguchi et al., 1990a,b; Tateno et al., 1996b; Alvarez et al., 1997; Kamiguchi and Tateno, 2002) after irradiation. The types of structural chromosomal aberrations and their incidence of one-cell embryos have been suggested to predict the genetic risk to the next generation in mice (Marchetti et al., 2004). However, several previous studies have shown that the incidence of structural chromosomal aberrations changes during the subsequent cleavages of one-cell mouse embryos exposed to either X-rays or neutrons (Weissenborn and Streffer, 1988a,b; Streffer, 1993) and two-cell mouse embryos exposed to X-rays (Weissenborn and Streffer, 1989). Supportive evidence was obtained with a micronucleus assay of two- to eight-cell mouse embryos exposed to fast neutrons at the one-cell stage (Pampfer et al., 1992).

In addition to increases in structural chromosomal aberrations during embryo development, a high incidence (24–52%) of hypoploid metaphases at the second and third mitoses of one-cell mouse embryos has been reported when the embryos were exposed to relatively low doses of X-rays (0.47–1.88 Gy) or neutrons (0.25–0.75 Gy) (Weissenborn and Streffer, 1988a,b). An increased incidence of aneuploid blastomeres (31.6%) was reported in eight-cell mouse embryos derived from the spermatozoa of males exposed to 4 Gy γ-rays (Mozdarani and Salimi, 2006). However, in these previous studies, the distinction between aneuploid embryos and mosaic embryos was imperfect, so it is unclear whether the high incidence of aneuploid metaphases accurately represents the frequent occurrence of aneupoid embryos. It is worth noting

that there was a significant increase in mosaicism, involving hypoploid and euploid cells, in 8.5-day postimplantation embryos when preovulatory mouse oocytes were exposed to 4 Gy X-rays (Tease and Fisher, 1996).

In the present study, we analyzed the chromosomes of mouse embryos at the first (one-cell), second (two-cell), and third (four-cell) cleavages of ova fertilized with spermatozoa that had been exposed to γ-rays to comprehensively assess the quantitative and qualitative changes in structural chromosomal aberrations, aneuploidy, and mosaicism during early cleavages. To distinguish between aneuploidy and mosaicism, we obtained metaphase spreads of all the sister blastomeres in two-cell and four-cell embryos in which the sister blastomeres had not separated. Furthermore, the kinetics of different types of γ-ray-induced structural chromosomal aberrations was investigated to understand the mechanism(s) underlying the development of aneuploidy and mosaicism.

Materials and methods

Animals

B6D2F1 (C57BL/6Cr×DBA/2Cr) hybrid mice (8–16 weeks of age) were purchased from Sankyo Labo Service Co. Inc. (Tokyo, Japan) and maintained under optimal conditions: light from 5:00 to 19:00 and room temperature at approximately 23°C. Laboratory animal diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given *ad libitum*. All experiments were performed according to the guidelines for animal experiments of our university.

Media

Organic and inorganic reagents were purchased from Nacalai Tesque Inc.

(Kyoto, Japan), unless specifically stated. Toyoda–Yokoyama–Hosi medium (TYH medium) was used for the in vitro manipulation of spermatozoa and oocytes (Toyoda et al., 1972). Chatot–Ziomek–Bavister medium modified by supplementation with 5.56 mM D-glucose (mCZB medium) was used to culture the embryos (Chatot et al, 1989). Both media were used at 37°C under 5% CO_2. The chemical compositions of both media have been previously reported (Tateno and Kamiguchi, 2007).

Exposure of spermatozoa to γ-rays and embryo production by in vitro fertilization (IVF)

Male mice were kept in suitably-sized cylindrical plastic tubes, and the testicular regions were exposed to a single dose of 2 Gy or 4 Gy ^{137}Cs γ-rays at a dose rate of 0.95 Gy/min. Within 24 h of irradiation, the spermatozoa were retrieved from the cauda epididymides and cultured in TYH medium for 1–1.5 h to induce capacitation. Three to five males were exposed to each dose. In some experiments, the spermatozoa from the same males were used to produce embryos at different developmental stages.

Female mice were intraperitoneally injected with 10 IU pregnant mare serum gonadotropin (PMSG; Teikoku-Zoki Pharmaceuticals, Tokyo, Japan), followed 48 h later by an injection of 10 IU human chorionic gonadotropin (hCG; Aska Pharmaceuticals, Tokyo, Japan) to induce superovulation. At 15–16 h after the hCG injection, the oocytes with cumulus cells were released from the oviducts into TYH medium. The oocytes were cultured with the capacitated spermatozoa for IVF. Two hours later, the oocytes were washed with mCZB medium and further cultured in the same medium.

Chromosome preparation and analysis

(1) One-cell embryos

At 6–8 h after insemination, the fertilized ova were transferred to mCZB medium containing 0.006 μg/mL vinblastine sulfate (Sigma-Aldrich, St. Louis, MO, USA) and cultured until they reached the first cleavage metaphase. At 18–20 h after insemination, the embryos were treated with 0.5% protease (commercially available as actinase E, Kaken Pharmaceuticals, Tokyo, Japan) in Dulbecco's phosphate-buffered saline for 6–8 min to loosen the zona pellucida. They were then kept in a hypotonic solution of a 1:1 mixture of 1% sodium citrate and 30% fetal bovine serum (FBS; Sigma-Aldrich) for 8–10 min at room temperature.

(2) Two-cell embryos

Approximately 32 h after insemination, two-cell embryos were transferred into mCZB medium containing both 0.01 μg/mL vinblastine sulfate and 3 μg/mL nocodazole (Sigma-Aldrich). This mixture of two different mitotic inhibitors was effective in spreading the chromosomes of the sister blastomeres at this embryonic stage. When the nuclei of both sister blastomeres became invisible, the embryos were treated with 0.5% protease to digest the zona pellucida. To avoid the separation of the sister blastomeres during the following hypotonic treatment, 10 μg/mL concanavalin A (Sigma-Aldrich) was added to the enzyme solution. The hypotonic treatment was performed in a 2:3 mixture of 1% sodium citrate and 40% FBS for 10 min at room temperature.

(3) Four-cell embryos

Approximately 42 h after insemination, four-cell embryos were transferred into mCZB medium containing 0.01 μg/mL vinblastine sulfate and cultured until the nuclei

175 of all the sister blastomeres had disappeared. As described above, the embryos were
176 placed in 0.5% protease solution containing 10 μg/mL concanavalin A to digest the zona
177 pellucida without separating the sister blastomeres. They were then kept in a hypotonic
178 solution (1:4 mixture of 1.2% sodium citrate and 60% FBS) for 10 min at room
179 temperature.
180
181 (4) Fixation, staining and analysis
182 The chromosomal slides of the embryos, regardless of their developmental
183 stage, were prepared with the gradual fixation–air drying method (Mikamo and
184 Kamiguchi, 1983). The slides were stained with 2% Giemsa (Merck KGaA, Darmstadt,
185 Germany) in phosphate-buffered saline (pH 6.8) (Mitsubishi Kagaku Iatron Inc., Tokyo,
186 Japan) for 8 min for conventional chromosome analysis. The slides were then processed
187 for C-band staining to differentiate the centromeric heterochromatin of the mouse
188 chromosomes except for the Y chromosome, as described elsewhere (Tateno et al.,
189 2000).
190 As illustrated in our previous paper (Tateno and Kamiguchi, 2007), structural
191 chromosome aberrations found in one-cell embryos were classified into seven
192 categories, i.e., chromosome break, chromosome gap, dicentric, translocation, ring,
193 chromatid break, chromatid gap and chromatid exchange. In addition to these categories,
194 deletions were scored in chromosome analysis of two-cell and four-cell embryos when
195 degenerative acentric fragments were observed. Acentric fragments of unknown origin
196 were scored as extra fragments.
197 Aneuploidy and mosaicism were identified by counting C-band positive
198 chromosomes. A dicentric chromosome was considered as consisting of two
199 centoromeric chromosomes. Degenerative chromosomes or lagging chromosomes in the

cleavage furrow were excluded from the centromere count.

When the metaphase spreads were incomplete owing to technical errors during slide preparation, the embryos were eliminated from structural and numerical chromosome analyses. Chromosome analysis of two-cell and four-cell embryos was limited to those embryos in which the metaphase spreads of all the sister blastomeres could be analyzed. Polyploid embryos arising from polyspermy were excluded from the data.

Statistical analysis

The chi-square test or Fisher's exact test was used to compare differences in the percentages of embryos with chromosomal aberrations. Differences in the frequencies of structural chromosomal aberrations per cell (blastomere) were analyzed with a nonparametric multiple comparison test. Differences were considered significant when $P < 0.05$.

Results

Development of mouse embryos derived from γ-irradiated spermatozoa

The rate of diploid one-cell embryos that reached the first cleavage metaphase was 100% in the 2 Gy group (n = 317), and 99.4% in the 4 Gy group (n = 352). The high developmental capacity of embryos after γ-irradiation was maintained at two-cell stage, because almost all the embryos reached metaphase after 2 Gy (98.9%, n = 186) or 4 Gy (98.2%, n = 222). The percentage of four-cell embryos, in which all the sister blastomeres reached metaphase, was still high in the 2 Gy group (96.4%, n = 197), although the percentage of these embryos in the 4 Gy group (93.7%, n = 190) was statistically ($p < 0.05$) lower than in the non-irradiated control group (97.9%, n = 285).

Overall, these results show no significant bias toward an underestimation of chromosomal damage attributable to developmental arrest in these cleavage embryos.

Structural chromosomal aberrations at each embryonic stage

As demonstrated in many previous studies, when spermatozoa exposed to γ-rays, the incidence of structural chromosomal aberrations was clearly enhanced in the resultant one-cell embryos (Table I). Most of these aberrations were of chromosome-type. In addition to the dramatic occurrence of chromosome breaks, the incidence of dicentric aberrations and translocations was significantly increased. Furthermore, there was a significant increase in chromatid breaks in the 2 Gy and 4 Gy groups and chromatid exchange in the 4 Gy group.

In the analysis of two-cell embryos, the embryos were scored as chromosomally abnormal when structural chromosomal aberrations were detected in at least one sister blastomere. As shown in Table I, the incidence of embryos with structural chromosomal aberrations was significantly higher in both irradiation groups than in the control group. Although the incidence of abnormalities at the two-cell stage was low in both irradiation groups compared with that at the one-cell stage, the difference was not statistically significant. The types of structural chromosomal aberrations found at the two-cell stage were similar to those at the one-cell stage, although deletions were newly apparent. From the one-cell stage to the two-cell stage, the incidence of chromosome breaks and dicentric aberrations decreased considerably, and chromatid breaks and exchanges became negligible (Figure 2).

In the chromosomal analysis of four-cell embryos, the control embryos displayed a relatively high incidence of structural chromosomal aberrations (Table I). Nevertheless, the incidence of embryos with structural chromosomal aberrations was

250 significantly elevated in both irradiation groups. When the net incidence of
251 radiation-induced structural chromosomal aberrations was calculated according to the
252 formula of Kamiguchi et al. (1990a), the aberration rates at the four-cell stage were
253 similar to those at the one-cell stage (Figure 1A). From the two-cell stage to the
254 four-cell stage, there was a reduction in dicentric aberrations and an increase in
255 chromatid breaks in the 2 Gy group, and an increase in chromosome and chromatid
256 breaks in the 4 Gy group. Certain of dicentric chromosomes and acentric fragments
257 evidently survived two cleavage divisions (Figure 2).
258
259 *Aneuploidy at each embryo stage*
260 In chromosome analysis at two-cell and four-cell embryonic stages, the
261 embryos were scored as hypoploidy when all sister blastomeres had hypoploid
262 metaphase, and as hyperploidy when all sister blastomeres had hyperploid metaphase.
263 There was no significant increase in aneuploidy in the one-cell embryos after both doses
264 of γ-rays (Table II), indicating that the irradiation of spermatozoa is not the primary
265 cause of aneuploidy. However, a significant increase in aneuploid embryos was
266 observed at the two-cell stage after irradiation. Hypoploid embryos were predominantly
267 observed. Interestingly, nearly half of them displayed degenerative or lagging
268 chromosomes in the cleavage furrow (Figure 3A). At the four-cell stage, the incidence
269 of aneuploidy in both irradiation groups was significantly higher than that in the control
270 group. However, the incidence declined from the two-cell stage to the four-cell stage
271 (Figure 1B).
272
273 *Mosaicism at each embryo stage*
274 Table III shows the incidence of mosaic embryos and the combination of sister

blastomeres with different chromosome numbers. At the two-cell stage, the incidence of mosaic embryos in the 2 Gy group was higher than that in the control group, but the difference was not statistically significant. The incidence increased significantly after irradiation with 4 Gy. All 17 mosaic embryos found in both irradiation groups had a hypoploid sister blastomere. There were degenerative chromosomes in 21.1% (4/19) of the hypoploid sister blastomeres (Figure 3B) and dicentric chromosomes in 80% (4/5) of the hyperploid sister blastomeres. At the four-cell stage, the incidence of mosaic embryos increased dose-dependently. A total of 65 mosaic embryos were found in both irradiation groups, and 60 (92.3%) had at least one hypoploid sister blastomere. In the 106 hypoploid blastomeres scored, 17% had degenerative chromosomes. Dicentric chromosomes were observed in 51.4% of the 35 hyperploid blastomeres. Some embryos still displayed degenerative chromosomes in the cleavage furrows. The net incidence of radiation-induced mosaicism increased stage-dependently (Figure 1C).

Discussion

In this study, over 90% of the one-cell embryos derived from irradiated spermatozoa developed to the four-cell cleavage metaphase. Therefore, we could detect unstable structural chromosomal aberrations and numerical chromosomal aberrations with a minimum of developmental arrest. However, the incidence of chromosomal deletions might have been underestimated in this study because this aberration type is difficult to detect with Giemsa and C-band staining. Balanced-type aberrations, such as reciprocal translocations and insertions, were not fully scored in these results because a fluorescent in situ hybridization (FISH) technique was not applied to the chromosome preparations. The incidence of these aberrations has been reported by Marchetti et al. (2004).

Our finding that the exposure of spermatozoa to γ-rays causes structural chromosomal aberrations in the resultant one-cell embryos is consistent with the results of previous studies. However, the incidence of structural chromosome aberrations in the present study was usually higher than that in the previous studies, even when the spermatozoa were irradiated with the same dose of γ-rays. For example, the aberration rate in one-cell embryos derived from spermatozoa following exposure to 4 Gy was 35.7% in the present study with B6D2F1 mice. This value is higher than the rates of 20.1% reported for B6C3F1 mice (Marchetti et al., 2004), 21.5% for C57BL/6J mice (Marchetti et al., 2007), and 14.7% for CBA×C57BLF1 mice (Tusell et al., 2004). In those studies, the spermatozoa were fertilized with oocytes within 7 days of irradiation, while the spermatozoa were used within 24 h of irradiation in the present study. However, radiation-induced sperm DNA damage can persist in maturing spermatozoa for at least 7 days before fertilization (Marchetti et al., 2007), because mammalian spermatozoa lack the ability to repair radiation-induced DNA damage (Sega et al., 1978; van Loon et al., 1991, 1993). Furthermore, it is unlikely that there were quantitative or qualitative differences in the radiation-induced sperm DNA damage among these mouse strains. In contrast, mammalian zygotes have the ability to repair DNA damage (Jaroudi and SenGupta, 2007), so sperm DNA damage can be repaired within the ooplasm after fertilization. When the repair proficient mouse strains were used, there were no strain-specific differences in the capacity of zygotes to repair the sperm DNA damage induced by ionizing radiation (Generoso et al., 1979; Derijck et al., 2008). Therefore, it is likely that the discrepancies in aberration rates between the present study and previous studies are largely attributable to the artificial loss of small acentric fragments during the fixation of the embryos.

Our results show that the incidence of radiation-induced structural

chromosomal aberrations in one-cell embryos fluctuated during subsequent cleavages (Figure 1A). Weissenborn and Streffer (1988a) also found that when mouse one-cell embryos were exposed to a higher dose range of X-rays (0.94-1.88 Gy) and neutrons (0.375-0.75 Gy) at 1 h post-conception, the aberration rates decreased from the first to the third mitosis after X-rays, whereas the aberration rates decreased at the second mitosis and increased at the third mitosis after neutrons. Furthermore, the investigators reported that the aberration rates decreased at the second mitosis and increased at the third mitosis in one-cell embryos exposed to X-rays (0.94 Gy) at 3 h, 6 h and 9 h post-conception (Weissenborn and Streffer, 1988b). A similar tendency was reported by Tusell et al. (2004), where the incidence of structural chromosomal aberrations at the two-cell stage was lower than that at the one-cell stage in mouse embryos after males exposed to 4 Gy X-rays were mated.

It has been suggested that the reduction in the incidence of radiation-induced structural chromosomal aberrations at the two-cell stage is attributable to the loss of acentric fragments during the first cleavage division, and that the increase in radiation-induced structural chromosome aberrations from the two-cell stage to the four-cell stage is attributable to the formation of new fragments (Weissenborn and Streffer, 1988a, b). In this study, the incidence of acentric fragments originating from chromosome and chromatid breaks, and dicentric chromosomes decreased considerably from the one-cell stage to the two-cell stage (Figure 2). These chromosomal losses would accounts for the reduction in the overall structural chromosome aberration rate in two-cell embryos. Chromosome analysis of four-cell embryos revealed that there were an increase in chromosome breaks/fragments in the 4 Gy group and an increase in chromatid breaks in both irradiation groups (Figure 2). The data may support the formation of new aberrations in postradiation cell cycles (Weissenborn and Streffer,

1988a, b).

In mouse preimplantation embryos, there are no functional G1/S and G2/M checkpoints in one- and two-cell stages, but S-phase checkpoint exist in one-cell stage. Apoptotic cells do not appear until morula stage in embryos derived from irradiated spermatozoa (Toyoshima, 2009). Derijck et al. (2006, 2008) demonstrated that phosphorylation of histone H2AX (γH2AX), which is maker for DNA double-strand breaks (DSBs), increased in remodeled male chromatin of mouse one-cell embryos derived from irradiated spermatozoa. Interestingly, Derijck et al. (2008) found that when the irradiation was performed during early S-phase of one-cell embryos, γH2AX foci were usually positioned in a single chromatid at the first mitotic metaphase. Adiga et al. (2007) and Yukawa et al. (2007) reported that when mouse pronuclear embryos from S-phase to G2-phase were exposed to γ-rays, γH2AX foci were detected at the four-cell stage. Thus, the previous results suggest that single strand DNA breaks, base damage or unrepaired DSBs in one-cell embryos can be persisted beyond cell divisions. This may be a reason why incremental appearance of chromosomal breaks was observed at the four-cell stage in embryos derived from irradiated spermatozoa in the present results. Recently, Ziegler-Birling et al. (2009) found higher levels of γH2AX during mitotic division of mouse four-cell and eight-cell embryos even in the absence of any induced DNA damage. They explained that γH2AX may play an important role in the chromatin remodeling during cleavage. It remains to be investigated whether levels of γH2AX is concerned with frequent occurrence of chromatid breaks/gaps at the four-cell stage in control embryos (Table I).

In this study, we found that the irradiation of spermatozoa markedly induced hypoploidy at the two-cell stage of the resultant embryos. Our chromosome preparations of cleavage embryos in which the sister blastomeres were not separated

allowed us to identify degenerative chromosomes that remained in the cleaving furrow of hypoploid embryos. These degenerative chromosomes probably came from the anaphase lag of aberrant chromosomes at the first cleavage division. This phenomenon is consistent with the observation of Weissenborn and Streffer (1988a,b). Therefore, it appears certain that anaphase lag is a leading cause of hypoploidy at the two-cell stage. The incidence of aneuploidy in our study was much lower than that in two- to four-cell mouse embryos exposed to lower doses of X-rays or neutrons (Weissenborn and Streffer, 1988a,b, 1989) and that in eight-cell mouse embryos derived from spermatozoa after exposure to 4 Gy γ-rays (Mozdarani and Salimi, 2006). In the present study, we found a significant increase in mosaic embryos consisting of hypoploid sister blastomeres, whereas no previous studies have noted the occurrence of mosaic embryos. It seems likely that the high incidence of hypoploidy reported in previous studies resulted from the frequent occurrence of the hypoploid blastomeres of mosaic embryos.

In a chromosomal analysis of mosaic embryos, we observed degenerative chromosomes in hypoploid blastomeres and dicentric chromosomes in hyperploid blastomeres. This strongly suggests that the loss of damaged chromosomes during the cell cycles of sister blastomere(s) and the nondisjunction of dicentric chromosomes between sister blastomeres during cleavage are the main mechanisms underlying the development of mosaicism in embryos derived from irradiated spermatozoa. There is supportive evidence to show that dicentric chromosomes induced in oocytes after exposure to X-rays at diakinesis survived two meiotic divisions and caused nondisjunction (de Boer and van der Hoeven, 1991). In our study, the incidence of mosaic embryos increased in γ-ray-dose- and embryo stage-dependent ways, whereas in contrast, the incidence of aneuploid embryos decreased from the two-cell stage to the four-cell stage (Figure 1). Tease and Fisher (1996) also found a significant increase in

mosaicism, involving hyperploid and euploid cells, in 8.5-day postimplantation mouse embryos derived from oocytes exposed to 4 Gy X-rays. These findings indicate that aneuploidy can eventually be converted to mosaicism during cleavage. Preimplantation genetic diagnosis of human embryos revealed that mosaicism was frequently generated through post-zygotic chromosome errors (Wells and Delhantry, 2000; Voullaire et al., 2000; Delhanty, 2005; Vanneste et al., 2009; Santos et al., 2010). Mosaic embryos were often accompanied with structural chromosome aberrations (Wells and Delhantry, 2000; Vanneste et al., 2009). Therefore, it is reasonable to consider a heritable risk of mosaicism rather than aneuploidy in embryos derived from spermatozoa after irradiation.

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Figure legends

Figure 1. Changing aspects of net incidences of embryos with γ-ray-induced structural chromosome aberrations (A), aneuploidy (B) and mosaicism (C) during early cleavages after 2 Gy (—▲—) and 4 Gy (—■—).

The net incidences were calculated according to the following formula of Kamiguchi et al. (1990a).

$$\text{Incidence of embryos with radiation-induced chromosome aberrations (\%)} = \left\{1 - \frac{\text{Number of embryos with a normal karyotype / Number of embryos analyzed (irradiated)}}{\text{Number of embryos with a normal karyotype / Number of embryos analyzed (control)}}\right\} \times 100$$

Figure 2. Fate of different types of γ-ray-induced structural chromosome aberrations during early cleavages after 2 Gy (—▲—) and 4 Gy (—■—). The net incidence of chromosome aberrations per cell (blastomere) was calculated according to the following formula of Kamiguchi et al. (1990a).

$$\text{Incidence of radiation-induced chromosome aberrations per cell} = \left\{1 - \frac{1 - \text{Number of chromosome aberrations / Number of embryos analyzed / 40* (irradiated)}}{1 - \text{Number of chromosome aberrations /Number of embryos analyzed /40* (control)}}\right\} \times 40^*$$

*40: the diploid number of the mouse.

Figure 3. Chromosome preparations of two-cell embryos derived from spermatozoa after γ-irradiation. **A**: Whole chromosome preparation showing two anaphase lagging chromosomes (arrows) in a cleavage furrow after 2 Gy. Owing to the loss of these chromosomes, the chromosome number of both sister

588 blastomeres is 38. pb: Nucleus of a second polar body. **B**: Metaphase spread
589 of one sister blastomere with a degenerative chromatin (arrow) after 4 Gy.
590 Bars indicate 20 μm.
591

Figure 1

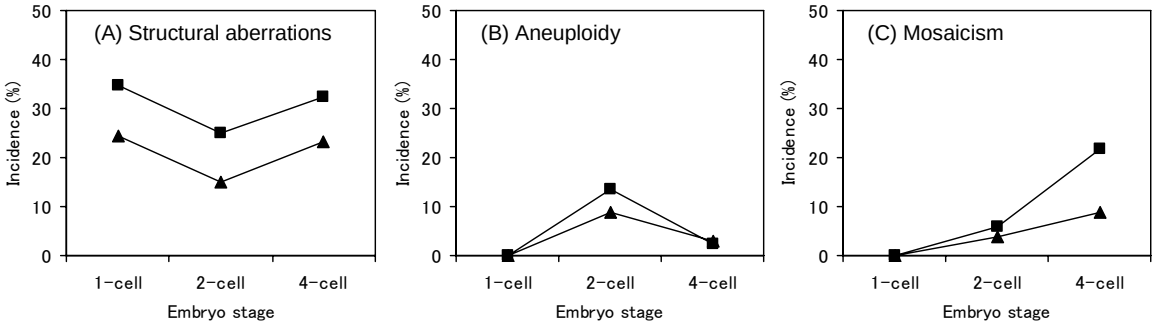
(A) Structural aberrations
(B) Aneuploidy
(C) Mosaicism
Incidence (%)
0
10
20
30
40
50
1-cell
2-cell
4-cell
Embryo stage

Figure 2

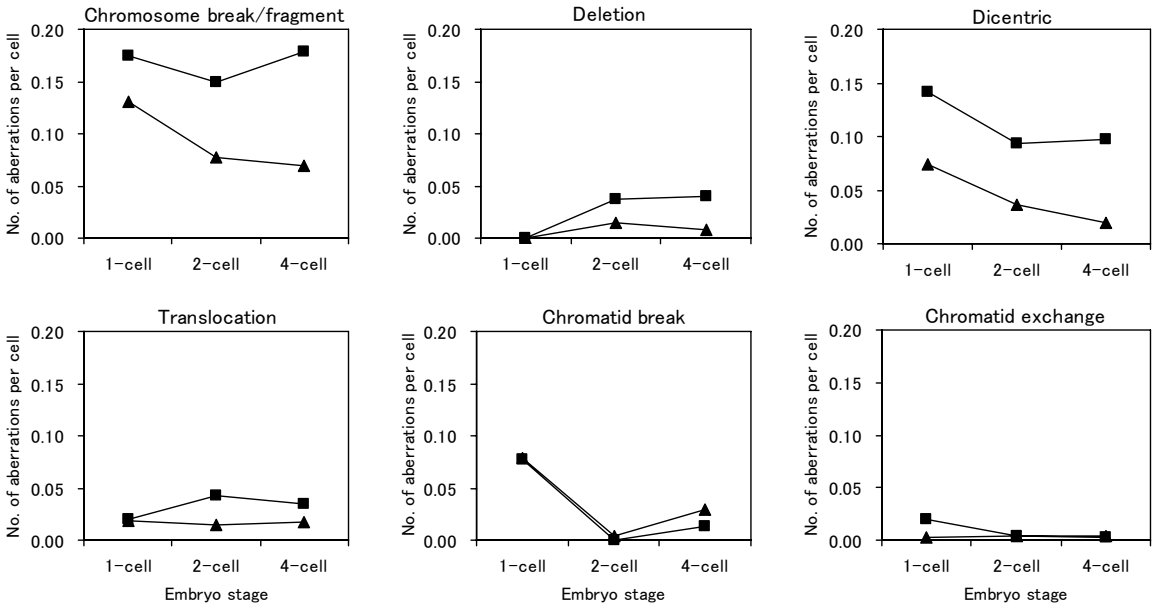
Chromosome break/fragment
Deletion
Dicentric
Translocation
Chromatid break
Chromatid exchange
No. of aberrations per cell
0.00
0.05
0.10
0.15
0.20
1-cell
2-cell
4-cell
Embryo stage

Figure 3

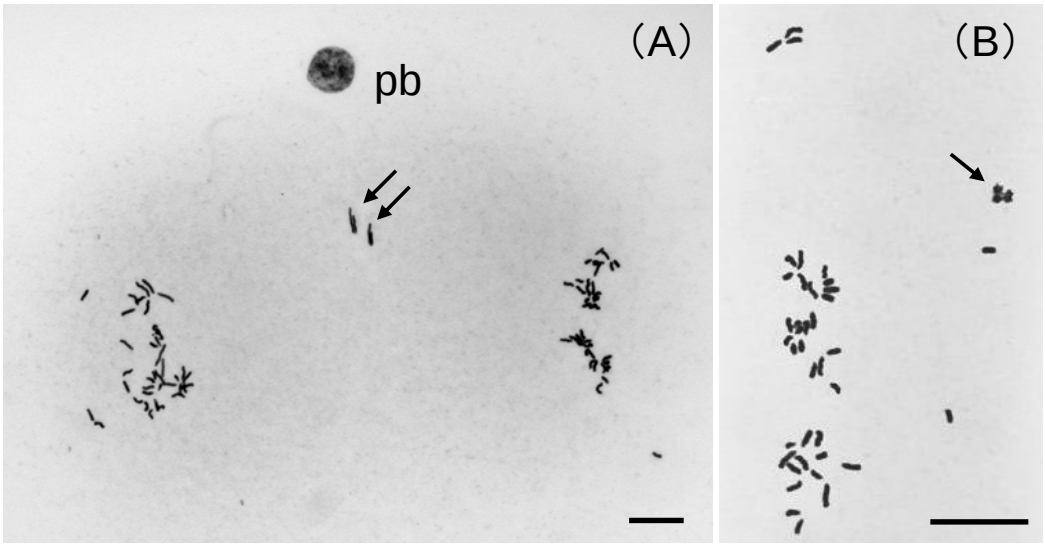

Table I Incidence of structural chromosomal aberrations at different developmental stages of mouse embryos derived from spermatozoa after γ-irradiation

Embryo stage	Dose (Gy)	No. of males used	No. of embryos analyzed	No. (%) of embryos with structural chromosomal aberrations	Total no. of structural chromosomal aberrations [per blastomere]	No. of different types of structural chromosomal aberrations [per blastomere]								
						Chromosome-type						Chromatid-type		
						break/ fragment	gap	dicentric	trans-location	deletion	ring	break/ fragment	gap	exchange
one-cell	0	6	503	8 (1.6)	8 [0.016]	4 [0.008]	0 [0.0]	1 [0.002]	0 [0.0]	0 [0.0]	0 [0.0]	3 [0.006]	0 [0.0]	0 [0.0]
	2	5	317	82 (25.9) [c]	105 [0.331] [c]	44 [0.139] [c]	2 [0.006]	24 [0.076] [c]	6 [0.019] [b]	0 [0.0]	0 [0.0]	27 [0.085] [c]	1 [0.003]	1 [0.003]
	4	4	350	125 (35.7) [c]	163 [0.466] [c]	64 [0.183] [c]	4 [0.011] [a]	50 [0.143] [c]	7 [0.020] [b]	0 [0.0]	1 [0.003]	29 [0.083] [c]	1 [0.003]	7 [0.020] [b]
Two-cell	0	8	187	5 (2.7)	7 [0.019]	2 [0.005]	0 [0.0]	2 [0.005]	0 [0.0]	0 [0.0]	0 [0.0]	3 [0.008]	0 [0.0]	0 [0.0]
	2	4	132	23 (17.4) [c]	45 [0.170] [c]	22 [0.083] [b]	0 [0.0]	11 [0.042] [a]	4 [0.015] [a]	4 [0.015] [a]	0 [0.0]	3 [0.011]	0 [0.0]	1 [0.004]
	4	4	133	36 (27.1) [c]	91 [0.342] [c]	41 [0.154] [c]	1 [0.004]	26 [0.098] [c]	11 [0.041] [b]	10 [0.038] [b]	0 [0.0]	1 [0.004]	0 [0.0]	1 [0.004]
Four-cell	0	5	200	52 (26.0)	74 [0.093]	9 [0.011]	1 [0.001]	1 [0.001]	1 [0.001]	2 [0.003]	0 [0.0]	40 [0.050]	19 [0.024]	1 [0.001]
	2	4	174	75 (43.1) [c]	161 [0.231] [c]	56 [0.080] [c]	2 [0.003]	14 [0.020] [b]	13 [0.019] [b]	7 [0.010]	2 [0.003]	56 [0.080] [a]	7 [0.010] [a]	4 [0.006]
	4	3	164	82 (50.0) [c]	290 [0.442] [c]	125 [0.191] [c]	2 [0.003]	65 [0.099] [c]	24 [0.037] [c]	28 [0.043] [c]	0 [0.0]	42 [0.064]	2 [0.003] [b]	2 [0.003]

[a,b.c] Significantly different from the non-irradiated control (0 Gy) in the same column: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$

Table II Incidence of aneuploidy at different developmental stages of mouse embryos derived from spermatozoa after γ-irradiation

Embryo stage	Dose (Gy)	No. of embryos analyzed	No. (%) of aneuploid embryos	hyperploidy	hypoploidy
One-cell	0	503	9 (1.8)	3	6
	2	317	5 (1.6)	1	4
	4	350	2 (0.6)	1	1
Two-cell	0	187	2 (1.1)	1	1
	2	132	13 (9.8)[b]	2	11
	4	133	19 (14.3)[b]	3	16
Four-cell	0	200	0	0	0
	2	174	5 (2.9)[a]	0	5
	4	164	4 (2.4)[a]	1	3

[a,b] Significantly different from the non-irradiated control (0 Gy) : [a]$P < 0.05$; [b] $P < 0.001$

Table III Incidence of mosaicism at different developmental stages of mouse embryos derived from spermatozoa after γ-irradiation

Embryo stage	Dose (Gy)	No. of embryos analyzed	No. (%) of mosaic embryos	Combinations of sister blastomeres with different chromosome numbers*				
				2*n*–/2*n*–	2*n*–/2*n*	2*n*–/2*n*/2*n*+	2*n*–/2*n*+	2*n*/2*n*+
Two-cell	0	187	3 (1.6)	0	2	-	1	0
	2	132	7 (5.3)	0	6	-	1	0
	4	133	10 (7.5)[a]	2	4	-	4	0
Four-cell	0	200	10 (5.0)	0	9	1	0	0
	2	174	23 (13.2)[a]	1	14	8	0	0
	4	164	42 (25.6)[b]	2	21	9	5	5

[a,b] Significantly difference from the non-irradiated control (0 Gy) : [a]$P < 0.01$; [b]$P < 0.001$

*2*n*–, 2*n* and 2*n*+ indicate hypoploidy, euploidy and hyperploidy, respectively.