Sister Chromatid Exchange Frequency in Early Embryos and Offspring Derived from IVF or ICSI in Mice

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Abstract: *To demonstrate chromosomal injuries associated with in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), sister chromatid exchange (SCE), an indicator of DNA damage, was investigated and the cytogenetic influence of embryonic manipulation on chromosomes of early embryos and offspring was investigated. SCE analysis was performed in early embryos and offspring obtained by IVF, ICSI, or in vivo fertilization (control). To label chromosomes of early embryos, the embryos were cultured in BrdU-supplemented medium for 2 cell cycles. For offspring, BrdU solution was repeatedly injected intraperitoneally. Chromosome samples prepared from the embryos and the bone marrow cells of offspring were stained by the Fluorescence plus Giemsa (FPG) method. In the IVF and ICSI groups, the rates of early development, implantation and offspring were lower than those in the control group. The SCE frequency of early embryos was significantly higher in the IVF and ICSI groups than in the control group ($P < 0.05$). In contrast, the SCE frequency of offspring in the IVF and ICSI groups was not significantly different from that in the control group. These findings suggest that embryos having serious DNA damage due to embryonic manipulation may be eliminated in a relatively early developmental step and may not reach term.*

Key words: *IVF, ICSI, Sister chromatid exchange, BrdU, DNA damage*

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Introduction

Recently, there has been rapid development in embryo engineering technology through advances in research into the use of mammalian germ cells. Artificial manipulation of gametes *in vitro* has been widely used in the field of human fertility treatment and is known as assisted reproductive technology (ART). *In vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), which are methods of manipulating and fertilizing gametes *in vitro*, are the two principal technologies utilized in ART. In particular, ICSI is an effective treatment in which gametes are fertilized by injecting only one spermatozoon or spermatid into the cytoplasm of an oocyte directly with a micromanipulator. This technology is useful for breeding domestic animals and in protecting endangered ones. However, the risk to manipulated embryos increases with the diversity and complexity of the technology, and there is a concern that these manipulated embryos lose their normality.

The safety of embryonic manipulation has not been thoroughly discussed. We tend to believe that embryonic abnormality has no immediate relationship to serious problems during pregnancy such as malformation and abortion. As a result, the study of the genotoxicity of preimplantation embryos is presently less advanced than that of postimplantation. However, it has been reported that the artificial manipulation of gametes *in vitro* during IVF and ICSI leads to high incidences of chromosomal abnormality [1–9]. In addition, the continual advancement of perinatal care may enable manipulated embryos to develop to term in the near future. Therefore, safety studies of embryonic manipulation are now being considered [1, 2].

The assessment of the frequency of sister chromatid

exchange (SCE) is a well-known procedure which is used for detecting DNA damage [10–18]. Because SCE is induced when chemicals or radiation that are carcinogenic, cytotoxic, or genotoxic affect cells, analysis of SCE is considered to be a sensitive indicator of DNA damage in cytogenetic studies [13–17, 19]. SCE is the complete exchange of double-stranded sister chromatid DNA that occurs at the same position of each sister chromatid. For eukaryotic cells, to overcome the potentially mutagenic and cytotoxic effects of double-stranded breaks (DSBs) generated spontaneously or by alkylating agents, the postreplicational DNA repair mechanism of DSBs is triggered. Papachristou *et al.* suggested that chromosomal abnormality was positively correlated with increased levels of DSBs, and proposed that SCE plays a crucial role in the homologous recombination repair pathway [18]. However, SCE does not necessarily demonstrate mutagenicity or chromosomal abnormality, though it may reflect the cytotoxic effects of various sorts of chemical agents as an indicator of DNA damage, and currently, the relationship between SCE and chromosomal abnormality has not been clarified.

SCE detection is also well-known as a reliable procedure for mutagenicity and carcinogenicity screening [13–20]. The exposure of a living body to a mutagen, such as mitomycin C (MMC), increases the SCE frequency (called the induced SCE). Therefore, induced SCE can be measured to determine the sensitivity and specific response to mutagens as the difference of DNA repair capacity [13, 16, 18, 19]. The measurement of induced SCE using a mutagenic substance is important in the evaluation of the genotoxicity of offspring derived from manipulated embryos. Thus, the SCE methodology can be used to quantitate genetic stability and evaluate chromosomal fragility [18].

The objective of the present study was to evaluate the cytogenetic normality of embryos and offspring that were derived from IVF and ICSI by measuring SCE and induced SCE in mice.

Materials and Methods

Animals

B6D2F$_1$ male (10–15 weeks old) and female (8–10 weeks old) mice were used for conventional IVF (conv. IVF) or ICSI. ICR female mice, 10–12 weeks old, were used as recipient animals for embryo transfer. The mice were maintained under controlled conditions of light (14 h light from 6:00 to 20:00; 10 h dark) and temperature (24 ± 2ºC). All procedures in these animal experiments were conducted according to the guidelines approved by the Animal Research Committee of Tokyo University of Agriculture.

Procedure of conventional IVF

To obtain oocytes, female mice were superovulated by an injection of 7.5 IU PMSG (Teikoku Zohki, Tokyo, Japan) followed by an injection of 5 IU hCG (Teikoku Zohki) approximately 48 h later. The cumulus-oocyte complexes (COCs) released by puncturing the ampulla of the oviduct at 15 h after the hCG injection were collected in TYH medium containing 4.0 mg/ml bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) under mineral oil (Sigma). Before insemination, spermatozoa were collected from one cauda epididymis and pre-cultured in TYH medium at 37ºC under 5% CO_2 in air for 1.5 h. Insemination was performed to give a final sperm concentration of 1 × 10^5 /ml in a droplet of TYH medium. Five hours after insemination, oocytes with two distinct pronuclei and a second polar body were considered fertilized. These fertilized oocytes were transferred into a droplet of KSOM medium supplemented with essential and non-essential amino acids (Gibco, Grand Island, NY) and 1.0 mg/ml BSA (mKSOM) [21, 22], and they were cultured at 37ºC under 5% CO_2 in air for 96 h.

ICSI Procedure

COCs were collected in the same way as in the conv. IVF procedure. Cumulus cells were removed from oocytes after treatment with 0.1% hyaluronidase (Sigma) in Hepes-mCZB medium [23, 24]. Cumulus-free oocytes were washed in hyaluronidase-free medium and cultured at 37ºC in 5% CO_2 in air prior to microsurgical procedures. Spermatozoa collected from one cauda epididymis were pre-cultured in Hepes-mCZB at 37ºC under 5% CO_2 in air for 10–15 min.

ICSI was performed in a manipulation chamber using a piezo-micromanipulator (PMM-110FU, Prime tech Ltd., Japan). One part of the sperm suspension containing activated spermatozoa was mixed thoroughly with a droplet of Hepes-mCZB containing 10% polyvinyl-pyrrolidone (PVP) (Sigma) in the chamber. A motile spermatozoon was sucked into the injection pipette, and the head was separated from the tail by applying piezo-electric pulses (intensity 3–4, speed 1). To perform ICSI, oocytes were transferred from the incubator to the droplet of Hepes-mCZB in the manipulation chamber. After penetration of the zona pellucida by applying piezo-electric pulses (intensity 2, speed 1), oocytes received a sperm head injection into

the cytoplasm with a minimal amount of sperm suspension medium.

Sperm-injected oocytes were maintained in the operation medium (Hepes-mCZB) for about 15–20 min and were then transferred into a droplet of mKSOM medium at 37ºC under 5% CO_2 in air. Five hours after sperm injection, oocytes with two distinct pronuclei and a second polar body were considered fertilized. The fertilized oocytes were cultured in mKSOM medium at 37ºC under 5% CO_2 in air for 96 h.

Preparation of embryos fertilized in vivo

After an hCG injection, females were caged overnight with males for mating. Females showing vaginal plugs were sacrificed and oocytes were recovered from the ampulla of the oviduct 20–22 h after hCG injection. The fertilized oocytes were transferred into a droplet of mKSOM medium and were cultured at 37ºC under 5% CO_2 in air for 96 h.

Embryo transfer to recipient females

ICR female mice were made pseudopregnant by mating with vasectomized males and used as embryo recipients. Three days after IVF or ICSI, 10–20 embryos at the morula and blastocyst stages were transferred to the uterine horns of the recipient females. Offspring, which were bred to 10–12 weeks of age, were examined for SCE.

SCE analysis of embryos

Some embryos, which were not transferred to recipient females, were analyzed for SCE. Late 8-cell embryos or morulae were cultured for 2 cell cycles (approximately 20–24 h) in mKSOM medium containing 1.4 × 10^{-5} M 5-bromo-2'-deoxyuridune (BrdU) (Sigma) in the dark. Four hours before the termination of the culture, embryos were transferred into a droplet of mKSOM containing 0.1 μg/ml demecolcine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and were cultured at 37ºC under 5% CO_2 in air. Chromosome spreads were prepared according to the gradual fixation-air drying method of Kamiguchi *et al.* [25, 26]. Staining for SCE was carried out by the fluorescence plus Giemsa (FPG) technique [27], with slight modifications (Fig. 1). The slides were stained for 15 min with 10 μM Hoechst 33258 solution, rinsed with distilled water, mounted in Sörensen's buffer (pH 8.04), and exposed to BL-B light for 10 min at 45ºC at a distance of 10 cm. The slides were then rinsed with distilled water and stained with 4% Giemsa solution (Merck KGaA, Darmstadt, Germany).

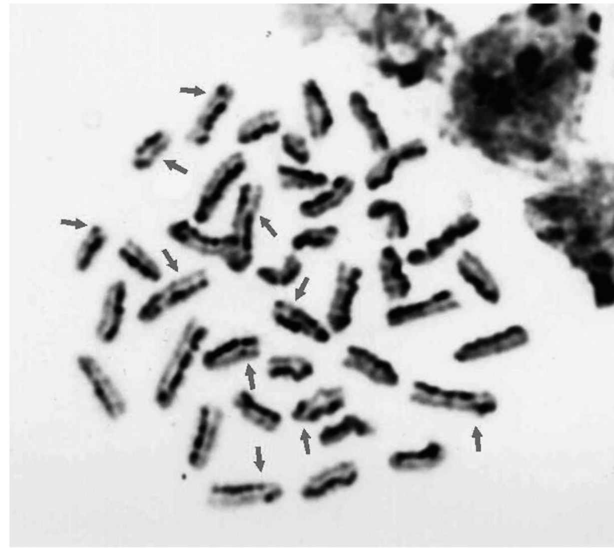

Fig. 1. Incidence of SCEs on a metaphase plate of a mouse embryo derived from ICSI (2n = 40). Double-stranded sister chromatid DNA labeled with BrdU is stained thinly by the FPG method. Arrows indicate the occurrence of SCEs on chromosomes.

SCE analysis of offspring

To detect SCE in bone marrow cells of the offspring, the mice were intraperitoneally injected 8–10 times of with BrdU solution (10 mg/kg wt, 0.1 ml each) at 1 h intervals. For the analysis of induced SCE, mitomycin C (MMC) (3 mg/kg wt, 0.1 ml) (Sigma) was given as a single intraperitoneal (i.p.) injection 15 min after the final BrdU injection. Instead of MMC, physiologic saline solution (0.1 ml) was administered to control animals in each experimental group. An i.p. injection of colchicines (10 mg/kg wt, 0.1 ml) (Sigma) was given 2 h before the animals were sacrificed. Eleven to 18 h after the final BrdU injection, bone marrow cells were collected from both femurs of the mice by washing out the bone marrow with 0.075M KCl (3 ml), and the cells were incubated at 37ºC for 30 min. The cells were then centrifuged (200 g) for 5 min and a fixative (3:1, methanol: glacial acetic acid) was added for 15 min at room temperature (22–25ºC). Following centrifugation (200 g, 5 min), 5 ml of fresh fixative was added, and the cells were kept in a freezer (–20ºC) overnight. On the next day, chromosome spreads were prepared by following the conventional procedure. The slides were stained in the same way as the slides for embryo analysis.

Table 1. *In vitro* development of mouse embryos derived from conventional IVF and ICSI

Group	No. of oocytes examined	No. (%) of oocytes fertilized	No. (%)* of embryos developing to		
			Blastocyst	Arrested	Degenerated
ICSI	1,025	964 (94.0)	846 (87.8)[a]	110 (11.4)	8 (0.8)
Conv. IVF	988	954 (96.6)	924 (96.9)[b]	28 (2.9)	2 (0.2)
Control	794	740 (93.2)	708 (95.7)[b]	24 (3.2)	8 (1.1)

*: % were calculated from the number of oocytes fertilized. Control group: embryos derived from *in vivo* fertilization. Percentages with different superscripts within the same column are significantly different ($P < 0.05$).

Table 2. The rates of implantation and offspring of mouse embryos derived from conventional IVF and ICSI

Group	No. of embryos transferred	No. (%)* of implantation site	No. (%)* of offspring
ICSI	102	39 (38.2)[a]	21 (20.6)[a]
Conv. IVF	74	46 (62.2)[b]	36 (48.6)[b]
Control	78	60 (76.9)[c]	48 (61.5)[b]

*: % were calculated from the number of embryos transferred. Control group: embryos derived from *in vivo* fertilization. Percentages with different superscripts within the same column are significantly different ($P < 0.001$).

Experimental design

This study consisted of two experimental sections: (1) SCE analysis of early embryos in the blastocyst stage and (2) SCE analysis of offspring derived from transferred embryos. In the embryo assay, embryos that were obtained after conv. IVF and ICSI treatment were examined as the conv. IVF and ICSI groups, respectively, and embryos that were fertilized *in vivo* were treated as the control group. In the offspring assay, SCE detection in all groups was performed with bone marrow cells under two conditions: spontaneous SCE, and SCE induced by the administration of MMC to the offspring.

Statistical analysis

In this study, we report the SCE frequency as "the percentage of SCE" or "the average of SCE". The percentage of SCE represents the proportion of SCE in all observed chromosomes, and the average of SCE indicates the SCE frequency in a metaphase plate as the mean ± standard deviation. The Chi-square test was used to determine the significance of the percentages of fertilization, embryo development, embryo implantation, offspring, and SCE. One-way ANOVA was used to compare the average of SCE among the groups.

Results

Full-term development of mouse embryos derived from IVF and ICSI

First, this study examined whether IVF and ICSI treatments could affect the fertilization rate and the early embryonic development. The results obtained are shown in Table 1. In the control, conv. IVF, and ICSI groups, 794, 988, and 1,025 oocytes, respectively, were used for examination, and 740 (93.2%), 954 (96.6%), and 964 (94.0%) of them were fertilized. There were no significant differences in fertilization rates among the three groups. The rates of embryonic development to morphologically normal blastocysts were 95.7% (708/740), 96.9% (924/954), and 87.8% (846/964) in the control, conv. IVF, and ICSI groups, respectively. The developmental rate of the ICSI group was significantly lower than those of the other two groups ($P < 0.05$). There was no significant difference between the control and the conv. IVF groups.

The results of embryo transfer to the recipient females are shown in Table 2. In the control, conv. IVF, and ICSI groups, 78, 74, and 102 embryos, respectively, were transferred to uteri of recipient females, and 60 (76.9%), 46 (62.2%), and 39 (38.2%) of them, respectively, were implanted. There were significant differences among all the groups ($P < 0.001$). The rates of transferred embryos reaching full-term development

Table 3. SCE frequency in mouse embryos derived from conventional IVF and ICSI

Group	No. of embryos analyzed	No. of m.p.* detected	No. of m.p. analyzed	No. of chromosomes analyzed	SCE incidence on chromosomes**		SCE (%)	SCEs/cell ± SD
					+	–		
ICSI	69	421	35	1,364	522	842	38.3[a]	14.9 ± 5.0[a]
Conv. IVF	47	209	54	2,135	460	1,675	21.5[b]	8.5 ± 3.7[b]
Control	45	198	50	1,984	285	1,699	14.4[c]	5.7 ± 2.9[c]

*: m.p. = metaphase plates, **: "+" or "–" shows whether SCE was detected or not. Control group: embryos derived from *in vivo* fertilization. Percentages with different superscripts within the same column are significantly different ($P < 0.001$).

Table 4. SCE frequency in mouse bone marrow cells derived from conventional IVF and ICSI

Group	MMC dose (3 mg/kg wt)	No. of offspring examined	No. of m.p.* analyzed	No. of chromosomes analyzed	SCE incidence on chromosomes**		SCE (%)	SCEs/cell ± SD
					+	–		
ICSI		3	37	1,471	196	1,275	13.3[a]	5.3 ± 1.4[a]
Conv. IVF	+	3	22	870	110	760	12.6[a]	5.0 ± 1.7[a]
Control		3	29	1,152	136	1,016	11.8[a]	4.7 ± 1.8[a]
ICSI		3	51	2,025	134	1,891	6.6[b]	2.6 ± 1.5[b]
Conv. IVF	–	3	31	1,238	86	1,152	6.9[b]	2.8 ± 2.0[b]
Control		3	42	1,658	90	1,568	5.4[b]	2.1 ± 1.9[b]

*: m.p. = metaphase plates, **: "+" or "–" shows whether SCE was detected or not. Control group: offspring derived from *in vivo* fertilization. Percentages with different superscripts within the same column are significantly different ($P < 0.001$).

were 61.5% (48/78), 48.6% (36/74), and 20.6% (21/102) in the control, IVF, and ICSI groups, respectively. The rate of the ICSI group was significantly lower than those of the other two groups ($P < 0.001$). However, there was no significant difference between the control and the conv. IVF groups.

SCE frequency of embryos

Forty-five, 47, and 69 embryos were used for chromosomal preparation in the control, conv. IVF, and ICSI groups, respectively. Of 198, 209, and 421 metaphase plates used for analysis, 50, 54, and 35 metaphase plates, respectively, were suitable for SCE analysis. The percentages of SCE, which show the proportions of SCE in all observed chromosomes in each group, are presented in Table 3. In the control group, of 1,984 chromosomes analyzed, 285 (14.4%) showed SCE occurrence. In contrast, 2,135 and 1,364 chromosomes were used for SCE analysis in the conv. IVF and ICSI groups, respectively, and 460 (21.5%) and 522 (38.3%) of them showed SCE. There were significant differences among all the groups ($P < 0.001$).

As shown in Table 3, the averages of SCE, representing the average of the number of chromosomes with induced SCE in a metaphase plate were 8.5 ± 3.7 and 14.9 ± 5.0 SCE/cell in the IVF and ICSI groups, respectively, and they showed significant differences from the average of SCE in the control group (5.7 ± 2.9 SCE/cell, $P < 0.001$). Additionally, the average of SCE in the ICSI group was significantly higher than that in the conv. IVF group ($P < 0.001$).

SCE frequency of bone marrow cells in offspring

SCE detection in all groups of the offspring was performed with the bone marrow cells under two conditions: spontaneous SCE, and SCE induced by the administration of MMC to offspring. The percentages of SCE in bone marrow cells of the offspring are presented in Table 4. Because the percentages of SCE in the MMC-administered groups were significantly higher than those of non-administered groups ($P < 0.001$), we consider that SCE was induced by the administration of MMC. Three mice in each group, which were derived from conv. IVF and ICSI embryos, were used for examination. For spontaneous SCE, 42, 31, and 51 metaphase plates were found suitable for analysis in the control, IVF, and ICSI groups, respectively. Of 1,658 chromosomes analyzed, SCE was detected in 90 (5.4%) chromosomes in the control group. A total of 1,238 and 2,025 chromosomes were used for analysis, and 86 (6.9%) and 134 (6.6%) of them showed SCE in the IVF and ICSI groups, respectively. There were no

significant differences among the groups. Twenty-nine, 22, and 37 metaphase plates in the control, IVF, and ICSI groups were analyzed for induced SCE. Of 1,152, 870, and 1,471 chromosomes detected, 136 (11.8%), 110 (12.6%), and 196 (13.3%), respectively, showed SCE. There were no significant differences among the groups.

The results of the SCE averages are also shown in Table 4. The MMC-administered and non-administered groups showed significant differences in SCE averages ($P < 0.001$). Spontaneous SCE numbered 2.8 ± 2.0 and 2.6 ± 1.5 SCE/cell in the IVF and ICSI groups, respectively, and there were no significant differences with the control group (2.1 ± 1.9). The average induced SCE was 4.7 ± 1.8, 5.0 ± 1.7, and 5.3 ± 1.4 in the control, IVF, and ICSI groups, respectively, and there were no significant differences among the groups.

Discussion

The influence of IVF and ICSI on the early development of mice was investigated using cytogenetics. No significant differences were detected in the oocyte fertilization rates among the control, IVF and ICSI groups. The rate of embryonic development to blastocysts in the IVF group was not significantly different from that in the control group, but this rate was significantly different in the ICSI group.

For ICSI, the oocyte membrane was penetrated by piezo-electric pulses, and a pipette was inserted deeply into the cytoplasm to inject a spermatozoon. This procedure may place a larger stress on oocytes than conv. IVF and the other microinsemination techniques. Placing the first polar body at the 12 o'clock or 6 o'clock position when puncturing oocytes is considered to be a factor affecting the success of ICSI, because in these positions influences on the spindle and chromosomes during cell division are avoided [24].

The spindle is a mitotic apparatus that distributes chromosomes during cell division, and damage to the spindle by micromanipulation may cause numerical chromosomal aberration associated with abnormal chromosome disjunction or may induce mosaic embryo expression. In a study comparing the mosaic expression rates of ICSI- and IVF-derived embryos, no significant difference was detected, and micromanipulation correctly positioning the positions of the first polar body and puncture site avoided influencing the spindle [29, 30]. In our experiment, the frequency of aneuploid nuclear plate was slightly higher in the ICSI group than in the IVF and control groups, but the difference was not significant (data not shown), suggesting that the influence of the ICSI procedure on the spindle and chromosomes was small.

In this study, the implantation rates of the IVF and ICSI groups were significantly lower than that of the control group. In humans, IVF and ICSI are the most widely used techniques of sterility treatment, but implantation rates are still low [4]. One reason is chromosomal aberration caused by DNA damage occurring during *in vitro* embryonic manipulation. Yoshizawa *et al.* reported that the conception rate of early embryos prepared by IVF was low, and the frequency of chromosomal aberration in these embryos was high [1, 2]. Moreover, according to some authors, chromosomal aberration in relatively early embryonic development is the reason for the low development rate of ICSI-derived embryos. Munne *et al.* [5] reported that early chromosome condensation occurred at a high frequency in non-fertilized eggs after ICSI and that egg activity generally occurred after ICSI. Yoshizawa [2] performed chromosomal analysis of human oocytes judged to be non-fertilized or developmentally arrested after ICSI. They found that many of these oocytes were arrested at the stage of pronucleus formation or earlier, not in the metaphase of the second meiotic division, and immature oocytes may have been used in ICSI. In our experiment, it was difficult to identify immature oocytes during the ICSI procedure because the first polar body might have degenerated.

The influence of sperm has been reported to be a cause of the low development rate of ICSI-derived embryos [28, 31]. Kishikawa *et al.* [28] performed ICSI on mouse oocytes using spermatozoa with abnormal head morphology and those killed by damaging the plasma membrane. They found that chromosomal aberration occurred at a high frequency in the metaphase of the first cleavage, and its frequency markedly increased with time after sperm death.

To study manipulated embryos, we assessed SCE in chromosomes, which is a sensitive indicator of DNA damage. The highest SCE frequency was detected in the ICSI group and it was significantly different from that of the control and IVF groups. A similar result was obtained for the developmental rate of the embryos. With regard to the application of ICSI to oocytes, as described above, the physical risk is larger than in IVF. In addition, the use of piezo-electric pulses may have injured the plasma membrane and chromosomes of not only the oocytes but also the spermatozoa. In the present study, sperm immobilization and head isolation were performed using a piezo-micromanipulator.

According to some reports, piezo-electric pulses for tail removal are thought to damage the sperm plasma membrane to some extent [32–34]. However, these reports suggest membrane damage of spermatozoa has beneficial effects on fertilization. In the present study, we were not able to clarify the difference between membrane damage from piezo-electric pulses and that arising from SCE.

The SCE frequency of the IVF group was significantly higher than that of the control group, although the developmental rate to blastocysts was similar, suggesting that DNA damage was frequently caused, even though the embryos seemed to have developed normally. There is a possibility that embryos fertilized by IVF and ICSI sustain DNA damage. This view is supported by the significant decreases observed in the implantation rates of IVF and ICSI embryos in comparison to the control group.

In reproductive medicine research, especially clinical studies directly focusing on the fertilization process, high frequencies of fetuses and newborns with chromosomal aberration have been reported [5]. Many such studies were concerned with aberration of the sex chromosomes and reported that abnormal meiosis and DNA damage of germ cells used in treatment were the causes [35, 36]. We investigated the normality of offspring obtained by IVF and ICSI with regard to DNA damage by measuring SCE in bone marrow cells. The percentage of SCE and the average of SCE in the IVF- and ICSI-derived offspring were not significantly different from those in the control group. The significantly higher SCE values for both the IVF and ICSI groups than the control group in the analysis of the embryos suggests that when serious DNA damage is sustained by embryo in the IVF or ICSI procedure, embryonic development may stop at a relatively early developmental step, resulting in the embryo not reaching term. The implantation and offspring rates of the IVF and ICSI groups were lower than those of the control group, offering further evidence in support of this view.

With regard to MMC-induced SCE, no significant difference was detected among the IVF, ICSI and control groups. It is known that the *in vivo* administration of a mutagen, such as MMC, increases the SCE value (called induced SCE) [13, 16, 19]. In this study, the SCE value was increased in all groups, indicating that no specific reaction to MMC (mutagen) occurred. The results of the SCE analysis of the embryos and offspring suggest that the embryos that sustained serious DNA damage during the IVF or ICSI procedure stopped developing at a relatively early developmental step and did not reach term. If embryos obtained by IVF or ICSI are selected in sterility treatment, it may lead to a low pregnancy or conception rate. When an embryo that passes this selection reaches term, the DNA damage to the offspring may be low.

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