Mutation Research : Genetic Toxicology and Environmental Mutagenesis (2008) 657(1):26-31.

Chromosome aberrations in mouse embryos and fetuses produced by assisted reproductive technology.

Tateno H.

Chromosome aberrations in mouse embryos and fetuses produced by assisted reproductive technology

Hiroyuki Tateno

Department of Biological Sciences, Asahikawa Medical College, Asahikawa 078-8510, Japan

Correspondence to: Dr. H. Tateno,

Department of Biological Sciences, Asahikawa Medical College

2-1 Midorigaoka-higashi, Asahikawa 078-8510, JAPAN

FAX: +81-166-68-2783

E-mail: htateno@asahikawa-med.ac.jp

Running title: Chromosome aberrations in assisted reproductive technology

Key words: intracytoplasmic sperm injection, embryo, fetus, structural chromosome aberration

Abstract

The occurrence of structural chromosome aberrations in mouse one-cell embryos produced by intracytoplasmic sperm injection (ICSI) with mature spermatozoa was dependent on the type of sperm incubation medium and sperm incubation time. When cauda epididymal spermatozoa were used following incubation in bicarbonate-buffered TYH medium for 0 h (no incubation) and 0.5 h, the chromosome aberration rates (6.9% and 7.4%, respectively) in the resultant embryos were significantly higher than that (2.3%) in the IVF embryos. However, when the spermatozoa were incubated for 2−2.5 h and 6 h in the same medium, the chromosome aberration rates were reduced to the IVF embryo level (3.8% and 4.3%, respectively). When spermatozoa incubated in Hepes-buffered H-mCZB and phosphate-buffered PB1 media were used for ICSI, chromosome aberration rates in embryos were significantly high (8.6−28.1%) and increased in a time-dependent manner. On the other hand, when immature testicular spermatozoa were incubated in those three media for 0.5 h and 6 h, the incidences of resultant embryos with structural chromosome aberrations ranged between 7.4% and 11.7%, and there was no medium- and time-dependent change in these aberration rates.

To evaluate transmissible risk of chromosome aberrations to offspring, two- or four-cell embryos derived from cauda epididymal spermatozoa were transferred into the oviducts of pseudopregnant females and chromosomes of live fetuses were examined on gestational day 16. One (2.0%) mosaic fetus was found when spermatozoa were incubated in TYH for 2−2.5 h, and there were four (6.7%) fetuses displaying a structurally abnormal karyotype when spermatozoa were incubated in H-mCZB for 2−2.5 h, indicating that structural chromosome aberrations generated in ICSI one-cell embryos are transmissible to offspring. The causal mechanism of structural chromosome aberrations in ICSI one-cell embryos is discussed in relation to the acrosomal plasma membrane cholesterol and the acrosome.

1. Introduction

Assisted reproductive technology, including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), has contributed to the great evolution in reproduction of humans and other mammalian species. In particular, the ICSI technique has contributed to the treatment of human male infertility which could not be relieved by conventional IVF technique. ICSI also plays a promising role in assisted reproduction of endangered species. However, the previous chromosome studies on mouse one-cell embryos have cast some doubt as to whether there is a potential risk of generating chromosome damage using the ICSI technique, because incidences of structural chromosome aberrations in embryos produced by ICSI with normal spermatozoa [1−8] were considerably high compared to those in embryos produced by conventional IVF technique [9−13], though mouse strains and chromosome staining methods used were not common among these studies (Table 1). Recently, we found that when cauda epididymal spermatozoa were incubated in bicarbonate-buffered medium for 2 h or more, the incidence of structural chromosome aberrations in the resultant ICSI one-cell embryos was reduced to the IVF one-cell embryo level. However, there was a time-dependent increase in aberration rates in ICSI embryos when the spermatozoa were incubated in Hepes- and phosphate-buffered media [14].

This paper outlines the previous findings that the occurrence of structural chromosome aberrations in ICSI one-cell embryos derived from cauda epididymal spermatozoa was dependent on the kind of sperm incubation medium and sperm incubation time [14], and then provides new information on chromosome aberrations in ICSI one-cell embryos derived from testicular spermatozoa and fetuses developed from ICSI embryos of cauda epididymal sperm origin.

2. Materials and methods

2.1. Media

As described earlier [14], mouse embryo culture medium (mCZB) modified by the addition of 5.56-mM D-glucose [15] was used for incubation of metaphase II oocytes before sperm injection and fertilized eggs after sperm injection. For incubation of spermatozoa, three kinds of media with a different buffer system, i.e. bicarbonate-buffered TYH, Hepes-buffered H-mCZB and phosphate-buffered PB1, were used throughout the study [14]. Oocyte preparation and sperm injection into oocytes were performed in H-mCZB. The mCZB and TYH were used under 5% CO_2 in air, and H-mCZB and PB1 were used under 100% air.

2.2. ICSI procedures

Figure 1 depicts the procedures of mouse ICSI. Spermatozoa were collected from the cauda epididymides or testes of male B6D2F1 mice, 7−12 weeks of age. Spermatozoa were incubated in a droplet (100 μl) of TYH, H-mCZB or PB1 under paraffin oil at 37 ºC for various times. Oocyte-cumulus complexes were collected from the oviducts of females of the same strain after stimulation by an intraperitoneal injection of 8−10 IU equine chorionic gonadotropin (eCG) followed 48 h later with an injection of 8−10 IU human chorionic gonadotropin (hCG). Oocytes were freed from cumulus cells by treatment with 0.1% hyaluronidase in H-mCZB for 3−5 min, thoroughly washed with mCZB and temporally retained in a droplet (100 μl) of the same medium under paraffin oil at 37 ºC.

A small amount (2−3 μl) of sperm suspension was transferred into a droplet (approximately 10 μl) of a medium supplemented with 10% (w/v) polyvinyl pyrrolidone (PVP) under paraffin oil. As described earlier [14], the kind of medium used for supplementation of PVP was determined by the type of medium used for sperm incubation. Oocytes were transferred from mCZB into a droplet

(approximately 10 μl) of H-mCZB under the paraffin oil. The droplets for spermatozoa and oocytes were put in the same chamber. For ICSI with cauda epididymal spermatozoa, highly motile spermatozoa with normal morphology were used. For ICSI with testicular spermatozoa, the normal morphology was checked for selection of spermatozoa under a microscope because testicular spermatozoa are immotile. Just before injection, the head was separated from the tail by applying a few piezo pulses. Immediately afterward, the sperm heads were individually injected into oocytes using a piezo-micromanipulator as described by Kimura and Yanagimachi [15]. Following the injection of sperm heads, oocytes were washed thoroughly with mCZB and transferred into a droplet (100 μl) of the same medium under paraffin oil at 37 ºC for further cultivation.

2.3. Chromosome analysis of one-cell embryos

Six to eight hours after ICSI, eggs were transferred into a droplet of mCZB containing vinblastine (0.02 μg/ml) and cultured until they reached the first cleavage metaphase. Metaphase eggs were treated with 0.5% protease to digest the zona pellucida and placed in hypotonic solution (1:1 mixture of 1% sodium citrate and 30% fetal bovine serum) for 8 min at room temperature. Chromosome slides of eggs were made by the gradual fixation-air drying method [16]. The chromosome slides were stained with 2% Giemsa solution for 8 min, followed by C-band staining [3].

2.4. Embryo transfer and fetal chromosome analysis

When oocytes injected with a sperm nucleus developed to two- or four-cell embryos, they were transferred into the oviducts of CD-1 females, 8−12 weeks of age, on the first day of pseudopregnancy. Surrogate females were killed on day 16 of pregnancy, and live fetuses were surgically obtained from the uteri. A piece of fetus skin was cultured in D-MEM (SIGMA) with 20%

fetal bovine serum for chromosome analysis. Two to five days later, the cultures were exposed to colcemid (0.05 μg/ml) for 2−3 h, and then cells were harvested with trypsin-EDTA solution, treated with hypotonic solution (0.075M KCl), and fixed with methanol-acetic acid (3:1) fixative. Chromosome slides were conventionally stained with 2% Giemsa solution for chromosome counts. Then, G-band patterns of chromosomes were obtained by the routine trypsin digestion method. Identification of G-banded chromosomes was carried out according to the nomenclature of mouse chromosomes [17]. If necessary, fluorescent in situ hybridization (FISH) analysis using mouse chromosome-painting probes (CAMBIO, Cambridge, UK) was carried out according to the provided protocol.

2.5. Statistical analysis

Percentage of embryos and fetuses with chromosome aberrations were compared using either the chi-square test or Fisher's exact probability test where appropriate. Differences were considered significant at $P<0.05$.

3. Results

3.1. Chromosome analysis of ICSI one-cell embryos derived from cauda epidydimal spermatozoa

Epididymal spermatozoa were incubated in TYH, H-mCZB and PB1 for 0.5 h, 2−2.5 h and 6 h before ICSI. To obtain additional information, spermatozoa were directly transferred into the PVP-supplemented medium from the epididymis without incubation (0 h) in the present study. More than 200 embryos were produced to measure the structural chromosome aberration rates in each experimental group. As shown in Figure 2, the incidence of one-cell embryos with structural

chromosome aberrations was greatly changed by the type of sperm medium and sperm incubation time. When spermatozoa were incubated in TYH for 0 h and 0.5 h, the incidences of resultant embryos with structural chromosome aberrations were 6.9% and 7.4%, respectively. Both were significantly (P<0.01, χ^2-test) higher than that (2.3%) of IVF one-cell embryos, which was estimated in the previous study [14]. However, when spermatozoa were incubated for longer time intervals in the same medium, aberration rates (3.8% for 2−2.5 h and 4.3% for 6 h) were reduced to the IVF embryo level. When H-mCZB was used for sperm incubation, the incidence of structural chromosome aberrations increased in a time-dependent manner (8.6% for 0 h, 10.0% for 0.5 h, 14.7% for 2−2.5 h, 17.3% for 6 h). When PB1 was used, the aberration rate (17.4%) at the 0 h-incubation was significantly (P < 0.01, χ^2-test) higher than the comparable findings in other media. However, the rate at the 0.5 h-incubation (11.8%) became close to those in other media. After that, the aberration rates noticeably increased in a time-dependent manner (17.2% for 2−2.5 h, 28.1% for 6 h). As previously presented [14], breaks of chromosome-type and dicentric aberrations were predominantly observed in ICSI one-cell embryos derived from spermatozoa following the incubation in H-mCZB and PB1 in spite of the incubation time.

3.2. Chromosome analysis of ICSI one-cell embryos derived from testicular spermatozoa

As with cauda epididymal spermatozoa, testicular spermatozoa were incubated in three different media for 0.5 h and 6 h. Table 2 gives the incidences of resultant embryos with chromosome aberrations. There was no significant difference in the incidence of chromosome aberration rates between both sperm incubation times in each medium. In addition, no significant difference was found among three media at the same incubation time. The data in this Table were compared with matched data on structural chromosome aberration rates in ICSI embryos derived from cauda epididymal spermatozoa (Figure 3). The aberration rates at 0.5 h-incubation were not influenced by the sperm

source and the medium type. However, the aberration rate at 6 h-incubation in TYH was significantly ($P<0.05$, χ^2-test) lower in ICSI embryos derived from cauda epididymal spermatozoa than in ICSI embryos derived from testicular spermatozoa, and the opposite was seen when H-mCZB and PB1 were used for sperm incubation ($P<0.01$ and $P<0.001$, χ^2-test, respectively).

3.3. Chromosome analysis of fetuses developed from ICSI embryos

On the basis of chromosome analysis of ICSI one-cell embryos derived from cauda epididymal spermatozoa, three different sperm culture conditions were set up to produce ICSI fetuses, i.e. the low-risk culture condition (TYH for 2−2.5 h), the moderate-risk culture condition (H-mCZB for 2−2.5 h) and the high-risk culture condition (PB1 for 6 h). As shown in Table 3, there were no chromosome aberrations in fetuses developed from IVF embryos. However, one fetus in the low-risk group had a mosaic consisting of normal cells (75%) and hyperploid cells (25%) with a Robertsonian translocation (Figure 4A). In the moderate-risk group, four fetuses displayed abnormal karyotype. The incidence (6.7%) was considerably high ($P=0.0614$, Fisher's exact probability test) compared to that in IVF fetuses. Of four abnormalities, two were reciprocal translocations (Figure 4B and 4C), one was a paracentric inversion (Figure 4D), and the other one was determined to be a distal deletion (Figure 4E) because there was no translocated chromosome in any of the metaphase spreads following FISH with the DNA probe. In the high-risk group, no fetus had an abnormal karyotype. In this group, however, the number of fetuses karyotyped was limited to 35 because 27 (43.5%) of 62 embryos transferred were lost due to pre- and postimplantation death. This suggests that chromosome aberrations generated in ICSI one-cell embryos derived from cauda epididymal spermatozoa after the incubation in PB1 for 6 h were lethal.

4. Discussion

Chromosome analysis of mouse ICSI one-cell embryos revealed that the incubation of cauda epididymal spermatozoa in TYH for at least 2 h before injection into oocytes effectively protected the resultant embryos against the generation of structural chromosome aberrations. However, the same incubation of the spermatozoa in H-mCZB and PB1 increased the incidence of structural chromosome aberrations in the resultant embryos in a time-dependent manner. The adverse effect of sperm incubation on chromosomes of resultant embryos was observed when spermatozoa were incubated in Hepes-buffered TYH medium [14]. So far as the previous results indicated (see Table 1), the incidence of ICSI one-cell embryos with structural chromosome aberrations was relatively low (3.8−4.1%) when spermatozoa were incubated in TYH for 1−1.5 h before ICSI [2, 8], and there was a tendency to be high (5.5−14.1%) when spermatozoa were suspended in H-mCZB for a short time (5−15 min) prior to ICSI [1, 4−7]. With the exception of one [1] of these studies, the aberration rates might have been underestimated because dicentric aberrations could not be detected by conventional Giemsa staining. Another important finding in the present study is that there was no sperm incubation medium- and sperm incubation time-dependent change in the incidence of structural chromosome aberrations in ICSI one-cell embryos derived from testicular spermatozoa. As discussed later, the difference in chromosomal vulnerability of ICSI one-cell embryos between sperm sources may provide a clue to understanding of the causal mechanism of chromosome damage in ICSI one-cell embryos.

Although there was a mosaic fetus in the low-risk group (sperm incubation in TYH for 2−2.5 h), it is unlikely the mosaic aberration had a direct relationship to the ICSI technique; it is more likely a sporadic event during *in vitro* embryo culture. However, there is little doubt that occurrence of reciprocal translocations, inversion and deletion in fetuses of the moderate-risk group (sperm incubation in H-mCZB for 2−2.5 h) is attributable to the sperm incubation conditions. This is the first evidence to show that chromosome aberrations generated in ICSI one-cell embryos are transmissible to offspring in the mouse. Although there have been a number of chromosome studies of human ICSI

pregnancies, it is difficult to make a precise interpretation of these data because male infertile factors are involved in the results. Bonduelle *et al*. [18] reported that the incidence of *de novo* structural chromosome aberrations (0.44%) was about two times as high as natural pregnancies (0.16-0.22%). It remains to be ascertained whether the increased incidence of structural chromosome aberrations in human ICSI pregnancies is related to the sperm medium type and sperm incubation time.

It is fundamental to understand why the short incubation of mouse spermatozoa in TYH increases the risk of generating structural chromosome aberrations in resultant embryos and their long incubation in the same medium adversely decreases the chromosomal risk, and why the long incubation of spermatozoa in H-mCZB and PB1 increases the chromosomal risk in a time-dependent manner. With regard to the former question, physiological and biochemical changes such as capacitation and acrosome reaction in mouse spermatozoa during the incubation in TYH should be considered. Mouse cauda epididymal spermatozoa have an intact acrosome that covers nearly two thirds of the sperm head. Moreover the cholesterol distinctively distributes in the plasma membrane over the acrosomal region [19−22]. In conventional IVF, these components never enter the oocyte because the cholesterol is dissociated from the acrosomal plasma membrane during sperm capacitation [20, 21, 23] and the acrosome breaks down immediately before oocyte penetration [24]. In ICSI procedures, however, the acrosomal plasma membrane cholesterol and the acrosome are introduced into the oocyte when uncapacitated spermatozoa are injected. It has been shown that the acrosome can alter sperm chromatin remodeling in the ooplasm following ICSI in the mouse [25], the pig [26] and the rhesus monkey [27−29]. In addition, mouse acrosome enzymes can potentially induce deformation and degeneration of oocytes [30], and the removal of sperm plasma membrane and acrosome before ICSI improves embryonic development in mice [31]. Because inhibition of topological rearrangement of DNA during sperm chromatin remodeling causes DNA lesions [32, 33], it is highly possible that the alteration of sperm chromatin remodeling by the acrosomal plasma membrane cholesterol and/or the acrosome can lead to structural chromosome aberrations of paternal

origin. TYH is widely used for mouse IVF program because it can effectively promote sperm capacitation and trigger the acrosome reaction [34, 35]. Once the cholesterol efflux and/or the acrosome collapse occur, sperm nuclei can successfully achieve chromatin remodeling in the ooplasm. This may be the reason why the incidence of structural chromosome aberrations is reduced in ICSI one-cell embryos derived from spermatozoa after a long incubation period in TYH. In other words, chromosome damage in ICSI one-cell embryos derived from uncapacitated spermatozoa after a short incubation in TYH, H-mCZB and PB1 may be due to the interference of sperm chromatin remodeling with the acrosomal plasma membrane cholesterol and/or the acrosome.

The fact that there was no time-dependent increase of structural chromosome aberration rates in ICSI one-cell embryos derived from testicular spermatozoa dissipated a doubt that H-mCZB and PB1 may be deleterious to mouse sperm DNA, and alternatively raised the possibility that a different causal mechanism may underlie the time-dependent increase of structural chromosome aberrations in ICSI one-cell embryos derived from cauda epididymal spermatozoa following the incubation in these media. Chromosomal studies of mouse one-cell embryos derived from *in vitro* aged spermatozoa found that the incidence of structural chromosome aberrations of paternal origin increased from 1% to 6% after 6 h of *in vitro* aging [36] and 12% after 12 h of *in vitro* aging [37] when spermatozoa were stored in unsupplemented Tyrode (T6) medium. Using sperm chromatin structure assay (SCSA), Estop *et al*. [36] indicated that *in vitro* incubation of mouse mature spermatozoa in unsupplemented T6 altered the chromatin structure, and they speculated that the subtle change of chromatin structure makes DNA susceptible to denaturation, thus leading to structural chromosome aberrations. It is generally accepted that the stability of sperm chromatin is dependent on the content of disulfide (−S-S−) bonds in nuclear protamines, and the greater the content of –S-S− bonds, the more stable the chromatin becomes. Since the –S-S− bonds in the nuclear protamines are exclusively formed during the transit of spermatozoa through the epididymis [38], cauda epididymal spermatozoa have a mature nucleus with high content of –S-S− bonds, while testicular spermatozoa

have an immature nucleus with low content of –S-S− bonds. There has been some evidence to support the idea that mature nuclei of epididymal spermatozoa are more resistant to physical and chemical stresses than immature nuclei of testicular spermatozoa [39−41]. However, the present results do not concur with the idea that the low content of –S-S− bonds in sperm nucleus can link to generation of structural chromosome aberrations, because the incidence of structural chromosome aberrations in ICSI one-cell embryos at the 6 h-incubation of spermatozoa in H-mCZB and PB1 was significantly higher when cauda epididymal spermatozoa were used versus when testicular spermatozoa were used.

It is possible that the acrosomal plasma membrane cholesterol may be involved in the time-dependent increase of structural chromosome aberrations in ICSI one-cell embryos derived from cauda epididymal spermatozoa following the incubation in H-mCZB and PB1. As described above, the cholesterol can be dissociated from the acrosomal plasma membrane during sperm capacitation. To induce sperm capacitation *in vitro*, appropriate concentrations of proteins, HCO_3^- and Ca^{2+} ions are essential. However, unsupplemented T6 used for *in vitro* aging of spermatozoa in previous studies [36, 37] lacks a protein source, H-mCZB includes no albumin and a low level of HCO_3^-, and PB1 lacks HCO_3^-. In these media, cauda epididymal spermatozoa inadequately undergo capacitation and retain a high level of acrosomal plasma membrane cholesterol. Therefore, it is speculated that when cauda epididymal spermatozoa are incubated under uncapacitated state, the residual cholesterol may undergo some alterations such as oxidation, thus increasing a risk of generating structural chromosome aberrations of paternal origin in ICSI one-cell embryos. Testicular spermatozoa may be insusceptible to incubation in H-mCZB and PB1 because they supposedly contain less cholesterol [42, 43]. It remains to be investigated whether the advanced removal of acrosomal plasma membrane cholesterol from cauda epididymal spermatozoa can protect the resultant embryos against chromosome damage and whether the distribution of cholesterol to the acrosomal plasma membrane of testicular spermatozoa can induce structural chromosome aberrations in the resultant embryos.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research (C): No.19591886 from the Ministry of Education, Culture, Sport, Science and Technology of Japan.

References

[1] H. Kishikawa, H. Tateno, R. Yanagimachi, Chromosome analysis of BALB/c mouse spermatozoa with normal and abnormal head morphology, Biol Reprod 61 (1999) 809−812.

[2] H. Kishikawa, H. Tateno, R. Yanagimachi, Fertility of mouse spermatozoa retrieved from cadavers maintained at 4 °C, J Reprod Fert 116 (1999) 217−222.

[3] H. Tateno, Y. Kimura, R. Yanagimachi, Sonication per se is not as deleterious to sperm chromosomes as previously inferred, Biol Reprod 63 (2000) 341−346.

[4] H. Kusakabe, M.A. Szczygiel, D.G. Whittingham, R. Yanagimachi, Maintenance of genetic integrity in frozen and freeze-dried mouse spermatozoa, Proc Natl Acad Sci USA 98 (2001) 13501−13506.

[5] M.A. Szczygiel, W.S. Ward, Combination of dithiothreitol and detergent treatment of spermatozoa causes paternal chromosomal damage, Biol Reprod 67 (2002) 1532−1537.

[6] H. Kusakabe, Y. Kamiguchi, Chromosome analysis of mouse zygotes after injecting oocytes with spermatozoa treated in vitro with green tea catechin, (−)-epigallocatechin gallate (EGCG), Mutat Res 564 (2004) 195−200.

[7] R. Suganuma, C.M. Walden, T.D. Butters, F.M. Platt, R.A. Dwek, R. Yanagimachi, A.C. van der Spoel, Alkylated imino sugars, reversible male infertility-inducing agents, do not affect the genetic integrity of male mouse germ cells during short-term treatment despite induction of sperm deformities, Biol Reprod 72 (2005) 805−813.

[8] H. Tateno, Y. Kamiguchi, How long do parthenogenetically activated mouse oocytes maintain the ability to accept sperm nuclei as a genetic partner?, J Assist Reprod Genet 22 (2005) 89−93.

[9] P.A. Martin-DeLeon, M.L. Boice, Sperm aging in the male after sexual rest: contribution to chromosome anomalies, Gamete Res 12 (1985) 151−163.

[10] Y. Matsuda, N. Seki, T. Utsugi-Takeuchi, I. Tobari, Changes in X-ray sensitivity of mouse eggs from fertilization to the early pronuclear stage, and their repair capacity, Int J Radiat Biol 55 (1989) 233−256.

[11] Y. Matsuda, I. Tobari, Repair capacity of fertilized mouse eggs for X-ray damage induced in sperm and mature oocytes, Mutat Res 210 (1989) 35−47.

[12] J. Santaló, J. Badenas, J.M. Calafell, V. Catalá, S. Munné, J. Egozcue, A.M. Estop, The genetic risks of in vitro fertilization techniques: the use of an animal model, J Assist Reprod Genet 9 (1992) 462−474.

[13] M. Yoshizawa, M. Takada, S. Nakamoto, T. Muramatsu, A. Okamoto, Analyses of developmental ability of (BALB/c × C57BL/6)F_1 and ICR mouse embryos fertilized in vitro and their chromosome at the first cleavage division, J Reprod Dev 39 (1993) 115−122.

[14] H. Tateno, Y. Kamiguchi, Evaluation of chromosomal risk following intracytoplasmic sperm

injection in the mouse, Biol Reprod 77 (2007) 336−342.

[15] Y. Kimura, R. Yanagimachi, Intracytoplasmic sperm injection in the mouse, Biol Reprod 52 (1995) 709−720.

[16] K. Mikamo, Y. Kamiguchi, A new assessment system for chromosomal mutagenicity using oocytes and early zygotes of the Chinese hamster, in: T. Ishihara, M.S. Sasaki (eds), Radiation-induced Chromosome Damage in Man, Alan R. Liss, New York, 1983, pp. 411−432.

[17] M.N. Nesbitt, U. Francke, A system of nomenclature for band patterns of mouse chromosomes, Chromosoma 41 (1971) 145−158.

[18] M. Bonduelle, E. Van Assche, H. Joris, K. Keymolen, P. Devroey, A. Van Steirteghem, I. Liebaers, Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters, Hum Reprod 17 (2002) 2600−2614.

[19] K. Toshimori, R. Higashi, C. Ōura, Distribution of intramembranous particles and filipin-sterol complex in mouse sperm membranes: Polyene antibiotic filipin treatment, Am J Anat 174 (1985) 455−470.

[20] Y. Lin, F.W.K. Kan, Regionalization and redistribution of membrane phospholipids and cholesterol in mouse spermatozoa during in vitro capacitation, Biol Reprod 55 (1996) 1133−1146.

[21] A.J. Travis, G.S. Kopf, The role of cholesterol efflux in regulating the fertilization potential of mammalian spermatozoa, J Clin Invest 110 (2002) 731−736.

[22] Y. Christova, P. James, A. Mackie, T.G. Cooper, R. Jones, Molecular diffusion in sperm plasma membranes during epididymal maturation, Mol Cell Endocrine 216 (2004) 41−46.

[23] P.E. Visconti, X. Ning, M.W. Fornés, J.G. Alvarez, P. Stein, S.A. Connors, G.S. Kopf, Cholesterol efflux-mediated signal transduction in mammalian sperm: Cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation, Dev Biol 214 (1999) 429−443.

[24] R. Yanagimachi, Mammalian fertilization, in: E. Knobil, J.D. Neill (eds), The Physiology of Reproduction, 2nd ed, Raven Press, New York, 1994, 189−317.

[25] A. Ajduk, Y. Yamauchi, M.A. Ward, Sperm chromatin remodeling after intracytoplasmic sperm injection differs from that of in vitro fertilization, Biol Reprod 75 (2006) 442−451.

[26] M. Katayama, M. Koshida, M. Miyake, Fate of the acrosome in ooplasm in pigs after IVF and ICSI, Hum Reprod 17 (2002) 2657−2664.

[27] P. Sutovsky, L. Hewitson, C.R. Simerly, M.W. Tengowski, C.S. Navara, A. Haavisto, G. Schatten, Intracytoplasmic sperm injection for rhesus monkey fertilization results in unusual chromatin, cytoskeletal, and membrane events, but eventually leads to pronuclear development and sperm aster assembly, Hum Reprod 11 (1996) 1703−1712.

[28] L. Hewitson, T. Dominko, D. Takahashi, C. Martinovich, J. Ramalho-Santos, P. Sutovsky, J. Fanton, D. Jacob, D. Monteith, M. Neuringer, D. Battaglia, C. Simerly, G. Shatten, Unique

checkpoints during the first cell cycle of fertilization after intracytoplasmic sperm injection in rhesus monkeys, Nat Med 5 (1999) 431−433.

[29] J. Ramalho-Santos, P. Sutovsky, C. Simerly, R. Oko, G.M. Wessel, L. Hewitson, G. Schatten, ICSI choreography: Fate of sperm structures after monospermic rhesus ICSI and first cell cycle implications, Hum Reprod 15 (2000) 2610−2620.

[30] K. Morozumi, R. Yanagimachi, Incorporation of the acrosome into the oocyte during intracytoplasmic sperm injection could be potentially hazardous to embryo development, Proc Natl Acad Sci USA 102 (2005) 14209−14214.

[31] K. Morozumi, T. Shikano, S. Miyazaki, R. Yanagimachi, Simultaneous removal of sperm plasma membrane and acrosome before intracytoplasmic sperm injection improves oocyte activation/embryonic development, Proc Natl Acad Sci USA 103 (2006) 17661−17666.

[32] D. Bizzaro, G. Manicardi, P.G. Bianchi, D. Sakkas, Sperm decondensation during fertilisation in the mouse: Presence of DNase I hypersensitive sites in situ and a putative role for topoisomerase II, Zygote 8 (2000) 197−202.

[33] H. Tateno, Y. Kamiguchi, Chromosome analysis of mouse one-cell androgenones derived from a sperm nucleus exposed to topoisomerase II inhibitors at pre- and post-fertilization stages, Mutat Res 556 (2004) 117−126.

[34] Y. Toyoda, M. Yokoyama, T. Hosi, Studies on the fertilization of mouse eggs in vitro: I. In vitro fertilization of eggs by fresh epididymal sperm (in Japanese), Jpn J Anim Reprod 16 (1971) 147−151.

[35] C.R. Ward, B.T. Storey, Determination of the time course of capacitation in mouse spermatozoa using a chlortetracycline fluorescence assay, Dev Biol 104 (1984) 287−296.

[36] A.M. Estop, S. Munné, L.K. Jost, D.P. Evenson, Studies on sperm chromatin structure alterations and cytogenetic damage of mouse sperm following in vitro incubation, Studies on in vitro-incubated mouse sperm, J Androl 14 (1993) 282−288.

[37] S. Munné, A. Estop, The effect of in-vitro ageing on mouse sperm chromosomes, Hum Reprod 6 (1991) 703−708.

[38] L.M. Bedford, H.I. Calvin, The occurrence and possible functional significance of –S-S− crosslinks in sperm heads, with particular reference to eutherian mammals, J Exp Zool 188 (1974) 137−156.

[39] K. Yanagida, R. Yanagimachi, S.D. Perreault, R.G. Kleinfeeld, Thermostability of sperm nuclei assessed by microinjection into hamster oocytes, Biol Reprod 44 (1991) 440−447.

[40] N. Kosower, H. Katayose, R. Yanagimachi, Thiol-disulfide status and acrigine orange fluorescence of mammalian sperm nuclei, J Androl 13 (1992) 342−348.

[41] T. Kaneko, D.G. Whittingham, J.W. Overstreet, R. Yanagimachi, Tolerance of the mouse sperm nuclei to freeze-drying depends on their disulfide status, Biol Reprod 69 (2003) 1859−1862.

[42] F. Suzuki, Changes in the distribution of intramembranous particles and filipin-sterol complexes

during epididymal maturation of golden hamster spermatozoa, J Untrastruct Mol Struct Res 100 (1988) 39−54.

[43] N. Seki, Y. Toyama, T. Nagano, Changes in the distribution of filipin-sterol complexes in the boar sperm head plasma membrane during epididymal maturation and in the uterus, Anat Rec 232 (1999) 221−230.

Figure legends

Figure 1. Mouse ICSI technique.

Figure 2. Change in incidence of structural chromosome aberrations in ICSI one-cell embryos derived from cauda epididymal spermatozoa following incubation in different media at various time intervals.

Figure 3. Comparison of structural chromosome aberration rates in ICSI one-cell embryos of testicular sperm origin and cauda epididymal sperm origin. Statistical significance for a vs.b: $P<0.05$, c vs. d: $P<0.01$ and e vs. f: $P<0.001$ using χ^2- test.

Figure 4. Chromosome aberrations found in fetuses developed from ICSI embryos derived from cauda epididymal spermatozoa. (A) Trisomy 16 with a Robertsonian translocation between chromosomes 3 and 16. (B) A reciprocal translocation between chromosomes 1 and 14. (C) A reciprocal translocation between chromosomes 2 and 3. (D) A paracentric inversion of proximal part of chromosome 12. (E) A distal deletion of chromosome 14. Opened brackets in B−E indicate the segment which is involved in the aberration.

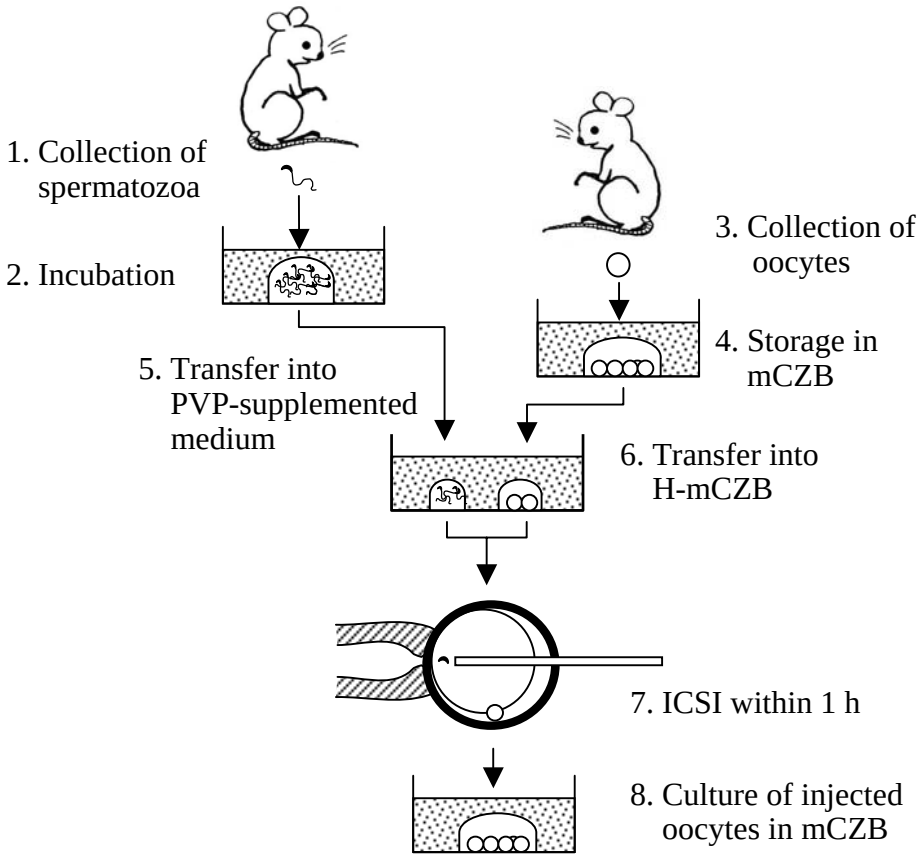

Figure 1
(Tateno)

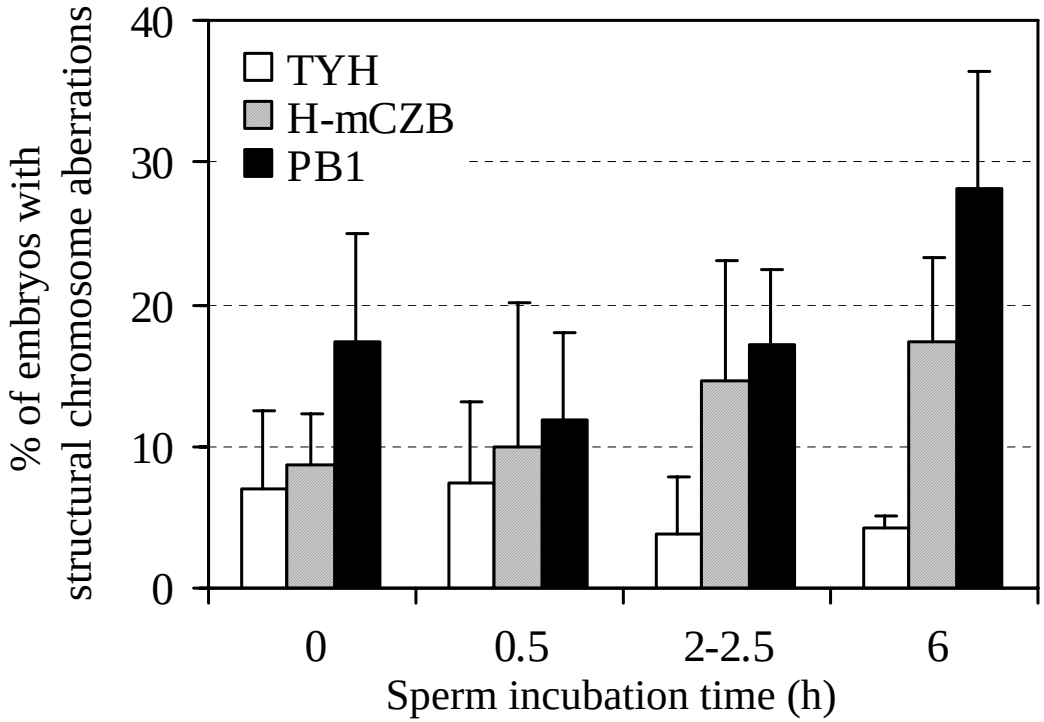

Figure 2
(Tateno)

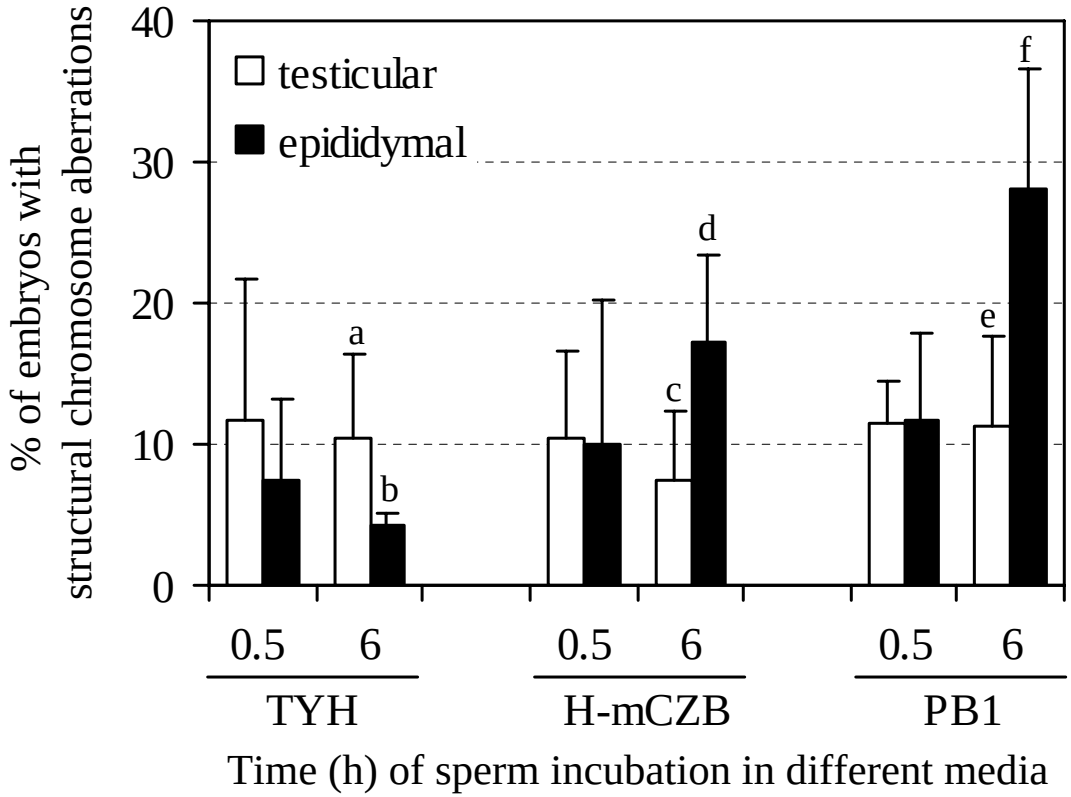

Figure 3
(Tateno)

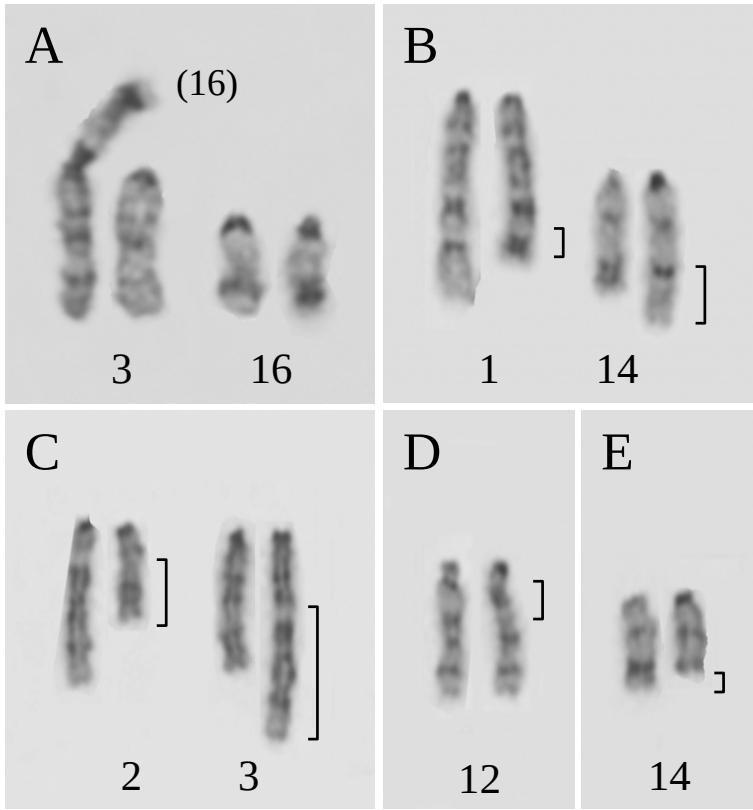

Figure 4
(Tateno)

Table 1. Previous studies on the incidences of structural chromosome aberrations in mouse 1-cell embryos produced by IVF and ICSI techniques.

Method for fertilization	Investigators	Genotype		No. of embryos analyzed	% of embryos with structural chromosome aberrations	Method for staining
		female	male			
ICSI	Kishikawa et al. [1]	(C57BL/6J × DBA/2J) F_1	BALB/c	184	14.1	C-band
	Kishikawa et al. [2]	(C57BL/6J × DBA/2J) F_1	(C57BL/6J × DBA/2J) F_1	98	4.1	C-band
	Tateno et al. [3]	(C57BL/6J × DBA/2J) F_1	(C57BL/6J × DBA/2J) F_1	85	9.4	C-band
	Kusakabe et al. [4]	(C57BL/6J × DBA/2J) F_1	(C57BL/6J × DBA/2J) F_1	145	7.6	Giemsa
	Szszygiel and Ward [5]	(C57BL/6J × DBA/2J) F_1	(C57BL/6J × DBA/2J) F_1	112	8.0	Giemsa
	Kusakabe and Kamiguchi [6]	(C57BL/6Cr × DBA/2Cr) F_1	(C57BL/6Cr × DBA/2Cr) F_1	91	5.5	Giemsa
	Suganuma et al. [7]	(C57BL/6J × DBA/2J) F_1	C57BL/6J	75	9.3	Giemsa
	Tateno and Kamiguchi [8]	(C57BL/6Cr × DBA/2Cr) F_1	(C57BL/6Cr × DBA/2Cr) F_1	157	3.8	C-band
IVF	Martin-Deleon et al. [9]	ICR	ICR	301	0.8	G- or Q-band
	Matsuda et al. [10]	(C57BL/6J × C3H/He) F_1	RFM	363	2.2	C-band
	Matsuda and Tobari [11]	(C57BL/6J × C3H/He) F_1	RFM	290	0.7	C-band
	Santalo et al. [12]	(C57B1/6J × CBA/Ca) F_1	(C57B1/6J × CBA/Ca) F_1	1033	1.7	C-band
	Yoshizawa et al. [13]	(BALB/c × C57BL/6) F_1	ICR	442	1.1	C-band
	Yoshizawa et al. [13]	ICR	ICR	454	1.3	C-band

Table 2. Chromosome analysis of mouse ICSI 1-cell embryos derived from testicular spermatozoa following incubation in different media for 0.5 h or 6 h.

Media for sperm incubation	Time (h) of sperm incubation	No. of experiments	No. of embryos analyzed	No. (%) of embryos with chromosome aberrations	
				structural aberrations	aneuploidy
TYH	0.5	9	205	24 (11.7)	4 (2.0)
	6	7	202	21 (10.4)	3 (1.5)
H-mCZB	0.5	7	209	22 (10.5)	2 (1.0)
	6	9	202	15 (7.4)	1 (0.5)
PB1	0.5	9	227	26 (11.5)	6 (2.6)
	6	10	212	24 (11.3)	3 (1.4)

Table 3. Chromosome analysis of live fetuses (gestational day 16) developed from ICSI embryos of cauda epididymal sperm origin.

Fertilization methods	Media for sperm incubation (incubation time)	No. of live fetuses karyotyped	No. (%) of fetuses with chromosome aberrations
IVF	TYH (1.5−2 h)	59	0
ICSI	TYH (2−2.5 h)	50	1 (2.0)
	H-mCZB (2−2.5 h)	60	4 (6.7)
	PB1 (6 h)	35	0