Production of offspring after sperm chromosome screening: an experiment using the mouse model

H. Watanabe1,*, H. Kusakabe1, H. Mori2, R. Yanagimachi3, and H. Tateno1

1Department of Biological Sciences, Asahikawa Medical University, Asahikawa 078-8510, Japan 2Department of Animal Science, School of Agriculture, Ibaraki University, Ibaraki 300-0332, Japan 3Institute for Biogenesis Research, University of Hawaii, Honolulu, HI 96822, USA

*Correspondence address. Tel: +81-166-68-2731; Fax: +81-166-68-2783; E-mail: h-wata@asahikawa-med.ac.jp

Submitted on February 6, 2012; resubmitted on August 17, 2012; accepted on October 9, 2012

STUDY QUESTION: Is it possible to produce offspring after sperm chromosome screening?
SUMMARY ANSWER: It is possible to produce zygotes after examining the genome of individual spermatozoa prior to embryo production.
WHAT IS KNOWN ALREADY: Chromosomal aberrations in gametes are a major cause of pregnancy loss in women treated with assisted reproductive technology. However, to our knowledge, there are no reports on the successful genomic screening of spermatozoa, although some attempts have been made using the mouse as a model.
STUDY DESIGN: To prevent the transmission of chromosomal aberrations from fathers to offspring, we performed sperm chromosome screening (SCS) prior to fertilization using the mouse as a model. The production of offspring after SCS consists of (i) replication of the sperm chromosomes, (ii) analysis of one copy of the replicated sperm chromosomes, (iii) construction of a zygote using another set of chromosomes and (iv) production of a transferable embryo.
MATERIALS, SETTING, METHODS: A single spermatozoon of a male mouse, with or without a Robertsonian translocation, was injected into an enucleated oocyte to allow the replication of sperm chromosomes. One of the sister blastomeres of a haploid androgenic 2-cell embryo was used for chromosome analysis. The other blastomere was fused with an unfertilized oocyte, activated and allowed to develop to a blastocyst before transfer to a surrogate mother.
MAIN RESULTS AND ROLE OF CHANCE: With high efficiency, we were able to analyze sperm chromosomes in a blastomere from the androgenic 2-cell embryos and culture zygotes, with and without aberrant chromosomes, to the blastocyst stage before embryo transfer. The karyotypes of the offspring faithfully reflected those of the blastomeres used for SCS.
LIMITATIONS, REASONS FOR CAUTION: This study was conducted using a mouse model; whether or not the method is applicable to humans is not known.
WIDER IMPLICATIONS OF THE FINDINGS: This study has shown that it is possible to produce zygotes without any paternally inherited aberrations by examining the genome of individual spermatozoa prior to embryo production.
STUDY FUNDING/COMPETING INTERESTS: This study was supported by a Grants-in-Aid for Scientific Research (22.8495 and 23890013 to H.W.) from the Japan Society for the Promotion of Science (JSPS). There are no conflicts of interest to be declared.

Key words: chromosome analysis / genetic diagnosis / sperm

Introduction
Some women who became pregnant after the application of assisted reproductive technology (ART) suffer from repeat spontaneous abortions. A major cause of pregnancy loss after ART, similar to that of spontaneous abortion after natural conception, is the presence of chromosomal aberrations in embryos that are inherited from the paternal and/or maternal gametes (Bettio et al., 2008; Martinez et al., 2010). The incidence of chromosomal aberrations in embryos may be higher when the fathers have abnormal karyotypes, such as...
47,XXY and 47,XY. Men with these karyotypes have a high risk of producing spermatozoa with sex chromosome aneuploidies. Furthermore, some infertile men without any chromosomal aberrations may produce aneuploid and/or diploid spermatozoa because of the occurrence of aberrant meiosis during spermatogenesis (Egozcue et al., 2000). In addition to numerical anomalies, structural chromosomal aberrations in spermatozoa are responsible for pregnancy loss (Zini et al., 2008).

Although preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) have been used (Franssen et al., 2011) to avoid aneuploidy-related spontaneous abortions, the clinical benefits of PGD/PGS remain uncertain (Harper et al., 2010; Franssen et al., 2011). PGD/PGS is an invasive method in which cleaving embryos or blastocysts receive microsurgical operation. Unless the blastocysts are frozen, blastocyst biopsy may not allow sufficient time for a detailed genetic diagnosis, as the blastocysts must be transferred to mothers by Day 6 of development. Furthermore, there are legal restrictions regarding the use of PGD/PGS in some countries. Thus, genetic screening of spermatozoa and oocytes prior to fertilization is a method that should be considered (Yanagimachi, 2011). The production of offspring after sperm chromosome screening (SCS) reported in this study consisted of four steps: (i) replication of the sperm chromosomes, (ii) analysis of one copy of the replicated sperm chromosomes, (iii) construction of a zygote using another set of chromosomes and (iv) production of a transferable embryo. To our knowledge, there are no reports on the successful genomic screening of spermatozoa, although some attempts have been made using the mouse as a model (Takeuchi et al., 2007, 2008).

Materials and Methods

Experimental design

The procedure used for SCS in this study is summarized in Fig. 1. Briefly, a spermatozoon was injected into an enucleated MII oocyte (Fig. 1A) to produce a haploid androgenic 2-cell embryo (Fig. 1B). One of the two sister blastomeres was fused with a zona-free MII oocyte to induce premature chromosome condensation (PCC) for the analysis of sperm chromosomes (Fig. 1C). The other sister blastomere was fused with another zona-free MII oocyte to construct a diploid zygote (Fig. 1D). This zygote was cultured until the blastocyst stage. Based on chromosome analysis of the first sister blastomere, embryos with known sperm chromosome constitutions were selected (Fig. 1E) and transferred to the surrogate mothers. Some androgenic blastomeres were cryopreserved in liquid nitrogen before fusion with zona-free MII oocytes for embryo production (Fig. 1F). We used the spermatozoa of male mice with a Robertsonian translocation to determine whether it is possible to preselect embryos with known chromosome constitutions prior to the embryo transfer.

Reagents and media

All chemicals were purchased from Nacalai Tesque, Inc. (Kyoto, Japan) unless otherwise stated. Oocytes/zygotes were cultured in a Chatot–Bavister (CZB) medium (Chatot et al., 1989) supplemented with 5.56 mM D-glucose and 5 mg/ml of bovine serum albumin (BSA, AlbuMax; GibcoBRL, Auckland, New Zealand). Collection and micromanipulation of oocytes were performed in the modified CZB medium supplemented with 20 mM HEPES-Na, 5 mM NaHCO3 and 0.1 mg/ml of polyvinyl alcohol (cold-water soluble; Sigma-Aldrich, St. Louis, MO, USA) instead of BSA (H-CZB). Spermatozoa were collected in the Toyoda–Yokoyama–Hosi (TYH) medium (Toyoda et al., 1971) supplemented with 20 mM HEPES-Na, 5 mM NaHCO3 and 0.1 mg/ml of polyvinyl alcohol instead of BSA (H-TYH). The pH of both H-CZB and H-TYH media was adjusted to ≚7.4. Dulbecco’s modified Eagle’s medium (D-MEM) supplemented with 10,000 IU of penicillin G potassium (Meiji Seika Pharmaceutical, Tokyo, Japan) and 10% fetal bovine serum (FBS; Cell Culture Technologies, Lugano, Switzerland) were used to culture the skin cells of offspring. CZB and D-MEM were used under 5% CO2 in air, and H-CZB and H-TYH were used under pure air.

Animals

Hybrid (C57BL/6 × DBA/2) F1 mice (BDF1; Japan SLC, Inc., Shizuoka, Japan) were used to collect oocytes and spermatozoa. ICR mice (Charles River Laboratories Japan, Inc., Yokohama, Japan) were used as surrogate mothers. Heterozygous carriers (ICR × C57BL/10) of a Robertsonian translocation between chromosomes 6 and 15 (Rb(6.15)) were also used as sperm donors because they innately provide spermatozoa with normal, balanced and unbalanced chromosome constitutions (Mori et al., 1995). All experiments were performed according to the Guideline for Animal Experiments of the Asahikawa Medical University.

Preparation of oocytes and spermatozoa for ICSI

Ovulation induction was induced in BDF1 females aged 7–11 weeks via intraperitoneal (i.p.) injection of 10 IU of eCG (Aska Pharmaceutical, Tokyo, Japan), followed by i.p. injection of 10 IU of hCG (Aska Pharmaceutical) 48 h later. Oocytes were recovered from the oviducts of treated mice between 14 and 16 h after hCG injection and immediately denuded of their cumulus cells in H-CZB containing 0.1% (w/v) hyaluronidase (Sigma-Aldrich). The cumulus-free oocytes were thoroughly washed with CZB and maintained in fresh CZB at 37°C until ICSI. Spermatozoa from the cauda epididymis of males aged 7–14 weeks were allowed to disperse in a droplet (5 μl) of H-TYH containing 10% polyvinyl pyrrolidone under paraffin oil (Merck Japan, Tokyo, Japan) in a Petri dish for ICSI.

Production of haploid androgenic embryos

Oocytes were enucleated in H-CZB containing 5 μg/ml of cytochalasin B (Sigma-Aldrich), as described elsewhere (Wakayama et al., 1998). Before sperm injection, a group of 15 oocytes was transferred to a droplet (5 μl) of H-CZB under paraffin oil, which had been placed next to a sperm-containing droplet in the same dish. A spermatozoon was aspirated into the injection pipette tail first, followed by separation of the tail from the head by application of a few piezo pulses. The heads were individually injected into enucleated oocytes according to the method of Kurate et al. (1996). Each series of ICSI experiments was completed within 1 h. ICSI oocytes were cultured in a droplet (100 μl) of fresh CZB under paraffin oil at 37°C until they reached the 2-cell stage.

Fusion of blastomeres and oocytes

At 24 h after ICSI, the androgenic 2-cell embryos were treated with 0.5% (w/v) protease (Kaken Pharmaceuticals, Tokyo, Japan) in calcium- and magnesium-free Dulbecco’s phosphate-buffered saline to digest the zona pellucida and separate the sister blastomeres. Concomitantly, fresh MI oocytes were successively treated with 0.1% hyaluronidase and 0.5% protease to remove the cumulus cells and the zona pellucida. One androgenic blastomere and one zona-free MI oocyte were then fused using the hemagglutinating virus of Japan (HVJ) Envelope Cell Fusion Kit (GenomeOne-CF; Ishihara Sangyo, Otsuka, Japan). Briefly, the MI
spindle of the recipient oocyte was located at the 9 o’clock position on a holding pipette attached to the micromanipulator and the HVJ envelope was sprayed on the surface of the oocyte at the 3 o’clock position using a fine glass pipette (20–25 μm in diameter). Subsequently, the zona-free oocyte was paired with a blastomere (Supplementary data, Movie S1). The paired oocytes/blastomeres were individually maintained for 30 min in CZB until complete fusion (Supplementary data, Movie S2). MII oocytes to be used for induction of PCC in blastomeres were maintained in medium containing the calcium-chelating agent BAPTA-AM (10 μM) (Sigma-Aldrich) to prevent their activation.

Culture of fused zygotes

The fused zona-free zygotes were maintained in CZB for 1.5–2 h at 37°C, and then transferred to calcium-free CZB containing 10 mM strontium chloride to allow their activation. Thirty minutes later, the zygotes were washed with CZB and individually cultured for 96 h in the same medium by placing them in a small depression in the surface of a culture dish, which was created using an aggregation needle (BLS, Budapest, Hungary). The development of each embryo was examined every 24 h.

Vitrification of blastomeres

Some androgenic blastomeres were cryopreserved in liquid nitrogen for 1–2 weeks using Cryotop (Kitazato BioPharma, Shizuoka, Japan) and a Vitrification Kit (VT101 and VT102, for vitrifying and warming, respectively; Kitazato BioPharma). Vitrifying and warming were performed according to the manufacturer’s instructions. After warming, blastomeres were cultured in CZB for 1.5–2 h and then used to construct fused zygotes, as described above.
Embryo transfer
Blastocysts were transferred into each oviduct of ICR females aged 8–14 weeks on the first day of pseudopregnancy to investigate fetal development. Recipients were sacrificed on Day 16 of pregnancy and the number of implantation sites and live fetuses was recorded. The fetuses were morphologically examined. For offspring delivery, blastocysts were also transferred into pregnant females. Offspring from transferred embryos were identified using hair color.

Preparation and analysis of chromosome slides
The fused zygotes were maintained in CZB for 2 h at 37°C to induce PCC in paternal chromosomes. The zygotes were then placed in hypotonic CZB (diluted 2-fold with pure water) for 10 min at room temperature. Chromosome spreads were prepared using the gradual-fixation/air-drying method (Mikamo and Kamiguchi, 1983). The slides were stained with 2% Giemsa (Merck) in buffered saline (pH 6.8) for 10 min. Some slides were subjected to conventional C-banding stain to identify centromeric heterochromatin. For the analysis of paternal chromosomes derived from spermatozoa of mice carrying a Robertsonian translocation, fluorescence in situ hybridization (FISH) was performed according to the manufacturer’s instructions to detect Rb(6.15) using DNA probes specific for chromosomes 6 and 15 (Applied Spectral Imaging, Migdal Haemek, Israel). After hybridization, slides were sealed with VECTASHIELD Mounting Medium containing DAPI (Vector Laboratories, Burlingame, CA, USA) before examination under a fluorescence microscope.

For chromosome analysis of offspring derived from the spermatozoa of carriers of Rb(6.15), skin cells from the tail tip were cultured in D-MEM for 3–5 days. The cells were then treated with 0.05 μg/ml of colcemid (GibcoBRL) for 2–3 h and then recovered by treating with 0.25% trypsin/1 mM EDTA solution. The cells were placed in a hypotonic 0.075 M KCl solution for 30 min at room temperature and fixed with a methanol/acetic acid (3:1) mixture. Chromosome spreads were air dried and subjected to FISH, as described above.

Statistical analysis
All experiments were performed at least three times. Statistical analyses were performed using logistic regression analysis using the JMP software (SAS Institute, Cary, NC, USA). Differences were considered significant at P < 0.05.

Results
Chromosome distribution in blastomeres of androgenones
The injection of spermatozoa into enucleated oocytes led to the successful development of 93.1% (162/174) to the 2-cell stage. Among them, 123 androgenones at the 2-cell stage (246 blastomeres) were used to determine whether the two blastomeres of each androgenone had identical chromosome constitutions. Chromosomes in two blastomeres of androgenones were examined after fusion of blastomeres with MII oocytes, with care being taken not to mix up blastomeres of two different androgenones. When blastomeres were fused with MII oocytes, 98.8% (243/246) of the zygotes were successfully fused. Then 230 fused zygotes (115 pairs) were fixed on glass slides and 218 zygotes had analyzable chromosomes originating from blastomeres at the G2 phase of the cell cycle (Fig. 2B–D). Among them, it was possible to analyze concurrently the chromosomes of

Figure 2
Chromosome spreads and morphological features of zygotes produced by fusion of androgenic haploid blastomeres with MII oocytes. (A) Chromosomal preparation of two fused zygotes derived from the two sister blastomeres of an androgenic haploid 2-cell embryo. Each zygote had a G2 prematurely condensed chromosome (G2-PCC) spread of blastomere origin (arrows) and an MII chromosome spread of oocyte origin (arrowheads). The broken line indicates the outline of fused zygotes. (B) Normal haploid (n = 20) chromosome spread of blastomere origin. (C) C-band staining of (B). (D) Structural chromosomal aberration of blastomere origin (arrow). (E) Diploid zygote with two pronuclei and two polar bodies. (F) Blastocysts derived from diploid zygotes after cultivation for 96 h. Bar = 100 μm.
104 pairs in both sister blastomeres. Chromosomes originating from the blastomeres and from the MII oocytes were easily distinguished based on their morphology (Fig. 2A); chromosome spreads originating from blastomeres were analyzed. As shown in Table I, 85 pairs (81.7%) had normal chromosomes in each blastomere; 9 pairs (8.7%) had chromosomes with identical abnormalities in two blastomeres and 10 pairs (9.6%) had normal chromosomes in one blastomere and abnormal chromosomes in the other blastomere. Together, ~90% of sister blastomeres in androgenones had identical chromosome constitutions.

Development of embryos that received haploid nuclei of androgenones

The developmental competence of MII oocytes fused with fresh or cryopreserved blastomeres from androgenones was investigated (Table II). In the case of fresh blastomeres, the fusion of 372 zona-free MII oocytes with a blastomere of a 2-cell androgenone and subsequent activation resulted in 284 (76.3%) zygotes that had two pronuclei and two polar bodies (Fig. 2E). They were considered as being ‘fertilized normally’. There were 52 zygotes with incomplete extrusion of polar bodies and 36 zygotes with unusually large polar bodies. These were considered as zygotes with abnormal ploidy and were discarded. Among the reconstructed zygotes, 53.8–63.6% developed to blastocysts and 22.6% of blastocysts that were transferred developed to live fetuses. Among 115 cryopreserved blastomeres, 110 (95.6%) were successfully defrosted, and 108 blastomeres were each fused with an MII oocyte to successfully produce 77 diploid zygotes (71.3%). Among these zygotes, 34 (44.2%) developed into blastocysts. This value was significantly lower (P < 0.05) than that of zygotes produced using non-frozen blastomeres (63.6%). However, the rates of implantation (47.1%) and fetal development (14.7%) after embryo transfer were similar to those of the zygotes produced using non-frozen blastomeres (42.9 and 22.6%, respectively).

Production of offspring after SCS

Haploid androgenic 2-cell embryos were prepared using the spermatozoa of males carrying a Robertsonian translocation. One blastomere of each embryo was used for chromosome analysis. FISH identified chromosome constitution as normal, balanced (Rb(6.15)) or unbalanced (Fig. 3). The other blastomere was used, without

---

**Table I** Homology of chromosome constitution in blastomeres of 2-cell androgenones.

<table>
<thead>
<tr>
<th>No. of 2-cell androgenones examined</th>
<th>Chromosome constitution (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal/normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal/abnormal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abnormal/abnormal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>85 (81.7)</td>
<td>10 (9.6)</td>
<td>9 (8.7)</td>
</tr>
</tbody>
</table>

*aChromosomal aberrations consisted of chromosome and chromatid breaks (eight and two cases, respectively).

*bChromosomal aberrations consisted of spermatozoan hypohaploidy, structural anomaly, and mosaicism (three cases each).

**Table II** Developmental competence of oocytes fused with androgenic blastomeres.

<table>
<thead>
<tr>
<th>Types of blastomere</th>
<th>No. of diploid zygotes constructed</th>
<th>No. (%) of zygotes developed to No. (%) of blastocysts transferred</th>
<th>No. (%) of Implantations</th>
<th>Live fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-cell</td>
<td>4-cell</td>
<td>8-cell/morula</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>130a</td>
<td>111 (85.4)</td>
<td>103 (76.2)</td>
<td>97 (74.6)</td>
</tr>
<tr>
<td></td>
<td>154b</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cryopreserved</td>
<td>77b</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*aZygotes were cultured to examine the development up to the blastocyst stage.

*bZygotes were cultured to produce transferable blastocysts.

*c-dValues without a common superscript were significantly different (P < 0.05).

---

**Figure 3** Fluorescence in situ hybridization (FISH) analysis of androgenic haploid G2 blastomeres derived from the spermatozoa of Rb(6.15) carriers. Chromosomes 6 and 15 were visualized using red and green colors, respectively. (A) Normal karyotype. (B) Balanced karyotype with Rb(6.15) (arrow). (C) Disomy of chromosome 15, including Rb(6.15) (arrowhead) and an extra chromosome 15 (arrow). (D) De novo reciprocal translocation involving chromosome 6 (arrows).
cryopreservation, for the production of offspring. Table III summarizes the results of experiments in which blastomeres with a normal chromosome constitution and those with a Rb(6.15) were separately fused with zona-free MII oocytes and allowed to develop to blastocysts before transfer to surrogate mothers. The karyotypes of the off-springs faithfully reflected those of the blastomeres used for SCS (Supplementary data, Fig. S1). Delivered offspring were all phenotypically normal and developed into fertile adults.

**Table III** Production of offspring after SCS.

<table>
<thead>
<tr>
<th>Karyotype of blastomeres used for production of offspring</th>
<th>No. of embryos transferred</th>
<th>No. (%) of offspring (sex)</th>
<th>No. (%) of offspring with Normal karyotype</th>
<th>Rb(6.15)</th>
<th>Unknown*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>66</td>
<td>18 (27.3) (1F, 13M)</td>
<td>16 (88.9)</td>
<td>0 (0)</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Rb(6.15)</td>
<td>22</td>
<td>5 (22.7) (2F, 3M)</td>
<td>0 (0)</td>
<td>5 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*aTwo offsprings could not be karyotyped because they were stillborn and their cells failed to grow during cell culture.

**Discussion**

In this study, we replicated chromosomes of a single spermatozoon, quickly analyzed one replicated chromosome set, constructed a zygote using the remaining set of sperm chromosomes and produced offspring with known male chromosome constitutions. We visualized sperm chromosomes by inducing PCC within a fresh MII oocyte. Prior to fixation of fused zygotes, the zygotes were treated with a hypotonic (1/2 diluted) CZB medium. This medium yielded better and more consistent chromosome spreads than did conventional hypotonic solutions, such as 1% sodium citrate (Araki et al., 2005) and a 1:1 mixture of 1% sodium citrate and 30% FBS (Tateno and Kamiguchi, 2007). Our chromosome analyses of androgenic 2-cell embryos revealed that 9.6% of the embryos had different chromosome constitutions in the two sister blastomeres. This may represent structural chromosomal aberrations generated artificially by the ICSI procedure. According to previous studies (Tateno and Kamiguchi, 2007; Watanabe et al., 2010a,b), 6–13% of ICSI mouse zygotes have unstable structural chromosomal aberrations. Unstable structural chromosomal aberrations occurring during cleavage after sperm irradiation have also been reported (Tateno et al., 2011). Nevertheless, the high homology (90.4%) in chromosome constitution between the two sister blastomeres of androgenic 2-cell embryos used here for chromosome analysis indicates that this embryonic stage is suitable for SCS. Prior to this study, we attempted to use haploid androgenic 4-cell embryos for SCS, because they provide four sister blastomeres instead of two, which would increase the reliability of the chromosome analysis. To our disappointment, sister blastomeres of haploid androgenic 4-cell embryos did not enter the G2 phase synchronously.

In this study, whole androgenic blastomeres at the G2 phase were fused with MII oocytes using an HVJ envelope to produce diploid zygotes, whereas in previous studies karyoplasts isolated from androgenic blastomeres were electrofused with parthenogenetically activated oocytes (Takeuchi et al., 2007, 2008). The delivery rate in this study was slightly higher than that observed in previous studies. Therefore, it seems unlikely that a mixture of cytoplasm between different cell stages affects the subsequent development of fused zygotes.

The results of SCS using spermatozoa of carriers of Rb(6.15) demonstrated that offspring could be obtained from sperm with preanalyzed chromosomes. This is the first report of successful SCS at the chromosomal level. Our success using a mouse model suggests that SCS may also be applicable to the spermatozoon of human translocation carriers. It may also be possible to apply advanced DNA technologies, such as array-comparative genomic hybridization and single-nucleotide polymorphism arrays, to genome-wide sperm genetic screening (SGS) (Harper and SenGupta, 2012). In addition, the technique of SCS could be used for sperm sexing in animal reproduction.

In this study, the two sister blastomeres of a haploid androgenic 2-cell embryo were concurrently fused with MII oocytes. One fused pair was used for chromosome analysis and the other was used for embryo production. Although SCS/SGS will become simpler and quicker with the advancement of technologies, it would be ideal to freeze blastomeres of androgenones until fully mature unfertilized oocytes become available. We found that blastomeres of androgenic 2-cell embryos from normal BDF1 male mice could be cryopreserved in liquid nitrogen using Cryotop (Kuwayama et al., 2005) and that zygotes constructed using cryopreserved blastomeres developed to live fetuses as well as did zygotes constructed using non-frozen blastomeres (Table II). Based on this result, we tried to perform SCS and embryo production using cryopreserved blastomeres from a carrier of Rb(6.15). However, the attempt was abortive because the resultant zygotes did not develop beyond the 2-cell stage. The genome of blastomeres from a carrier of Rb(6.15) may be more vulnerable to freezing compared with that of blastomeres from normal BDF1 mice. In any case, the cryopreservation of blastomeres certainly facilitates the SCS approach proposed here.

To conclude, this study has demonstrated that it is possible to produce mouse zygotes without any paternally inherited aberrations by examining the genome of individual spermatozoon prior to embryo production. These zygotes were able to develop normally and produce live offspring.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

**Acknowledgements**

The authors thank Dr Y. Fukui (Obihiro University of Agriculture and Veterinary Medicine) for his invaluable technical advice.
Authors’ roles
H.W. performed all experiments and drafted the paper. H.K. performed the FISH analysis. H.M. managed the Rb(6.15) mice. R.Y. and H.T. designed the study and drafted the paper.

Funding
This study was supported by a Grants-in-Aid for Scientific Research (22.8495 and 23890013 to H.W.) from the Japan Society for the Promotion of Science (JSPS).

Conflict of interest
None declared.

References
Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008;23:2663–2668.