Feasibility study of repeated fluorescent in-situ hybridization in the same human blastomeres for preimplantation genetic diagnosis

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In order to increase the number of chromosomes examined in each blastomere, we have developed a repeated fluorescent in-situ hybridization (FISH) procedure by which six or more chromosomes can be analysed per blastomere of a human embryo. Three consecutive FISH procedures with directly-labelled fluorescent Vysis DNA probes were carried out for examination of chromosomes X, Y, 11, 13, 18 and 21 in the same blastomeres (n = 126) and lymphocytes (n = 164). Based on the initial number of nuclei, the percentages of nuclear loss and presence of signals were 3 and 92% respectively in blastomeres; 6 and 91% respectively in lymphocytes after the first FISH; 7 and 87% respectively in blastomeres and 10 and 86% respectively in lymphocytes, after the second FISH. These percentages were 13 and 78% respectively in blastomeres and 14 and 81% respectively in lymphocytes after the third FISH. The FISH procedure was repeated successfully in a couple for preimplantation genetic diagnosis of chromosomal aneuploidies in biopsied blastomeres of their embryos in our clinic. In conclusion, it is feasible to carry out repeated FISH procedures in the same blastomeres. Six or more chromosomes of a single blastomere may be examined using this procedure.

Key words: fluorescent in-situ hybridization/gender determination/in-vitro fertilization/preimplantation genetic diagnosis

Introduction

Currently, fluorescent in-situ hybridization (FISH) is a common technique used in preimplantation genetic diagnosis (PGD) for the gender determination of an embryo or for screening for the most common trisomies. Human blastomeres and polar bodies have been analysed in PGD using FISH in which two or three DNA probes were often used (Griffin et al., 1992; Delhanty et al., 1993, 1997; Grifo et al., 1994; Harper and Delhanty, 1996; Harper et al., 1996; Verlinsky et al., 1996; Laverge et al., 1997; Lissens and Sermon, 1997). FISH with five fluorescence DNA probes was recently reported for analysis of five chromosomes in a single human blastomere (Munné and Weier, 1996; Gianaroli et al., 1997). However, because of the limited number of fluorescent colours of DNA probes, the number of chromosomes that can be examined simultaneously in each blastomere is limited to a maximum of five. In order to increase the number of chromosomes examined in each blastomere, we have developed a repeated FISH procedure by which six or more chromosomes can be analysed per blastomere of a human embryo.

Materials and methods

This study was approved by the Institutional Ethics Committee of the Greater Baltimore Medical Centre, USA.

Study 1

Human embryos (2–8-cell stage) obtained from oocytes with three pronuclei (3PN) after in-vitro insemination were used in the investigational experiments. For experiments, we prefer to use all blastomeres of an embryo. Therefore, we used isolation procedures for a single blastomere as described previously (Liu et al., 1993). Briefly, in this procedure, the zona pellucida was first dissolved in acid Tyrode’s solution. The zona-free embryo was then incubated in a Ca2+- and Mg2+-free medium for disassociation of the blastomeres. When a single blastomere was isolated, it was transferred into a droplet of HEPES-buffered human tubal fluid medium (H-HTF medium; Irvine Scientific, Santa Ana, CA, USA). The nucleus of the blastomere was carefully examined under an inverted microscope at ×400 magnification. Only those blastomeres that had a clear single nucleus in each blastomere were used in this study.

Study 2

The repeated FISH procedures were tested using human lymphocytes from fresh male peripheral blood obtained from a finger prick. Blood (50 µl) was taken and transferred into an Eppendorf tube containing 100 µl of a red cell lysing solution (Sigma R1129, St Louis, MO, USA). After mixing, the tube was centrifuged at 300 g for 5 min and the supernatant was removed. The pellet was resuspended in 100 µl H-HTF and centrifuged again at 300 g for 5 min. After centrifugation, the supernatant was removed and the pellet was then resuspended in ~50 µl H-HTF. A Petri dish was prepared as follows: several droplets of 30 µl H-HTF were put in the centre of the dish. Cell suspension (5 µl) was added to one of the droplets in the dish. A single lymphocyte was aspirated from the drop containing lymphocytes by using a mouth-control glass pipette and rinsed in the other cell-free droplets under an inverted microscope at ×200 magnification. The single lymphocyte was then put onto a glass slide. The slides were
Repeated FISH in single blastomeres

Figure 1. Repeated fluorescent in-situ hybridization (FISH) with whole chromosome print (WCP) probes in a single blastomere and lymphocyte. 1–3, the same nucleus of a blastomere that was from a three pronuclear embryo: the nucleus with (a) one green signal (for chromosome X) and one orange signal (for chromosome Y) after the first FISH; (b) two green signals (for chromosome 13) and four orange signals (for chromosome 21) after the second FISH and (c) two green signals (for chromosome 11) and two orange signals (for chromosome 18) after the third FISH. (d, e, f), the same nucleus of a lymphocyte that was isolated from a male: the nucleus with (d) one green signal (for chromosome X) and one orange signal (for chromosome Y) after the first FISH; (e) two green signals (for chromosome 13) and two orange signals (for chromosome 21) after the second FISH and (f) two green signals (for chromosome 11) and two orange signals (for chromosome 18) after the third FISH.

Table I. Results of repeated fluorescent in-situ hybridization (FISH) of the same blastomeres and lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Blastomeres (n = 126)</th>
<th>Lymphocytes (n = 164)</th>
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<tbody>
<tr>
<td></td>
<td>No. of nuclear loss from each FISH (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. nuclei with signals (%)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>First FISH</td>
<td>4 (3%, 3/126)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>116 (92%, 116/126)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Second FISH</td>
<td>5 (7%, 9/126)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>109 (87%, 109/126)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Third FISH</td>
<td>7 (13%, 16/126)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>98 (78%, 98/126)&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

There were no statistically significant differences of the rates of nuclear loss and nuclei with signals between blastomeres and lymphocytes.

<sup>a</sup>Percentage of total nuclear loss from initial number of blastomere nuclei.
<sup>b</sup>Percentage of nuclei with signals from initial number of blastomere nuclei.
<sup>c</sup>Percentage of total nuclear loss from initial number of lymphocyte nuclei.
<sup>d</sup>Percentage of nuclei with signals from initial number of lymphocyte nuclei.

then kept at room temperature for 45 min prior to FISH. The FISH procedures in lymphocytes were the same as those of the blastomeres.

Clinical applications

A couple (wife aged 39 years and husband aged 40 years) had a history of two abortions for chromosomal abnormalities of the fetuses after amniocentesis and karyotype analyses: one fetus had monosomy X (45,X) and was aborted at the 20th week of pregnancy, and the other had trisomy 21 and was aborted in the second trimester. They went through a cycle of in-vitro fertilization (IVF); the details of ovarian stimulation and oocyte retrieval in our centre were the same as previously described (Liu et al., 1997). At ~16 h after insemination, the oocytes were carefully examined under an inverted microscope for fertilization. Normal fertilization was assumed to have occurred when two clearly distinct pronuclei (2PN) were observed. After another 48 h of in-vitro culture, single blastomeres were removed from embryos with fair morphological quality (more than 4-cell stage with <40% volume of anucleate fragments) for PGD.

For the clinical case of PGD, only one or two blastomeres were removed from an early cleavage-stage embryo by micromanipulation for analysis. The procedure of embryo biopsy combining zona drilling and aspiration of the blastomeres was similar to that reported by Handyside et al. (1989). Briefly, using micromanipulation procedures an embryo was immobilized by a holding pipette. A hole of ~35–40 µm was made in the zona pellucida using acid Tyrode’s solution (Sigma) which had been aspirated in a 10 µm diameter micropipette.
Once the hole had been made in the zona pellucida, the pipette was removed and a blastomere was aspirated through this hole into a biopsy micropipette 35–40 µm in diameter. After biopsy, the holding and biopsy pipettes were gently withdrawn and the biopsied embryo was transferred into a 30 µl droplet of B2 medium under mineral oil (Sigma) and incubated at 37°C in an atmosphere of 5% CO2.

Blastomeres were examined using the repeated FISH procedures. Prior to FISH, blastomeres were fixed on glass slides using the procedure described by Coonen et al. (1994). Briefly, for fixation, a single blastomere was put in a premarked circle on a glass slide (Surgipath®; Richmond, IL, USA). A solution of 0.1% Tween 20 in 0.01 N HCl was used to remove the cytoplasm of the blastomere using a mouth-control glass pipette until the nucleus was free of cytoplasm.

**Fluorescent in-situ hybridization**

Three consecutive FISH procedures were carried out in each nucleus of a blastomere. The details of the repeated FISH in the same nuclei were the same as reported previously (Liu et al., 1998). In this study, for the first FISH, directly-labelled Vyssis whole chromosome point (WCP) probes (Vyssis, Downers Grove, IL, USA) were used to analyse chromosomes X and Y in the blastomeres. Denaturation of DNA and

### Table II. Fluorescent in-situ hybridization (FISH) results in embryos from oocytes with three pronuclei

<table>
<thead>
<tr>
<th>No. of embryos examined</th>
<th>No. of blastomeres</th>
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Once the hole had been made in the zona pellucida, the pipette was removed and a blastomere was aspirated through this hole into a biopsy micropipette 35–40 µm in diameter. After biopsy, the holding and biopsy pipettes were gently withdrawn and the biopsied embryo was transferred into a 30 µl droplet of B2 medium under mineral oil (Sigma) and incubated at 37°C in an atmosphere of 5% CO2. Blastomeres were examined using the repeated FISH procedures. Prior to FISH, blastomeres were fixed on glass slides using the procedure described by Coonen et al. (1994). Briefly, for fixation, a single blastomere was put in a premarked circle on a glass slide (Surgipath®; Richmond, IL, USA). A solution of 0.1% Tween 20 in 0.01 N HCl was used to remove the cytoplasm of the blastomere using a mouth-control glass pipette until the nucleus was free of cytoplasm.
hybridization were carried out at 78°C for 4 min and 37°C for 30 min respectively in an automatic slide baker (HYBrite, Vysis). After rinsing, the slides were air-dried, 12 μl of mixed solution of 1.25 ng/ml 4’, 6-diamidino-2-phenylindole (DAPI; Sigma) and Vectashield mounting medium (Vector Laboratories Inc, Burlingame, CA, USA) (1:1 v/v) were added to each of the slides and covered with a 22×22 mm coverslip (Richard-Allan Scientific, Richland, MI, USA). The coverslip was not sealed. The slides were evaluated under a fluorescence microscope (Zeiss Axioskop, Oberkochen, Germany) at ×1000 magnification. Signals of the first FISH were captured and stored in a computer using multicolour fluorescence imaging software (Biological Detection Inc, Pittsburgh, PA, USA). The main advantage of this software is that it does not allow the addition or deletion of any signals from FISH images.

For the second FISH, the slide with the same nucleus examined by the first FISH was rinsed sequentially for 10 min in each solution of 60% formamide/2× sodium chloride/sodium citrate (SSC), 2× SSC and 4× SSC/0.05% Tween 20 in a 50°C waterbath. The coverslip usually became detached from the slide and fell into the jar when the slide was rinsed in the solutions (removal of the coverslip by hand often damages the nucleus). The slides were then dehydrated sequentially in 70, 90 and 100% ethanol at room temperature. The second FISH procedure was the same as the first FISH, but using different chromosome probes: Vysis WCP probes 13 and 21 were used in the second FISH. Signals of the first FISH were captured and stored in a computer in the same way as in the first FISH.

When the evaluation of the second FISH was completed, the slides were again rinsed and a third FISH procedure was carried out on the same nucleus. The third FISH procedure was carried out using Vysis WCP probes 11 and 18 using the same protocol as in the second FISH. All three FISH procedures took ~6 h, depending on the number of nuclei examined. When the three FISH procedures were completed, the results were evaluated by examining the colours and locations of signals of probes from the three FISH procedures.

**DNA probe control**

The control was used to check whether the DNA probes or solutions used in the FISH procedure were functioning normally. A few drops of fresh human peripheral blood were taken from a finger prick and used to make smears on glass slides. The FISH procedure on the blood samples was the same as that of the first FISH procedure on blastomeres. Each newly purchased probe was first tested using the blood samples. For each test, 100 interphase nuclei of a blood sample were counted in each slide. The rates of absent signal and present signals were examined in these 100 nuclei. A probe was considered to be acceptable if the positive signal rate was >90% in the control.

**Evaluation of the results**

In studies 1 and 2, nuclear loss was defined as the absence of the nucleus on the slide after FISH. A present signal was defined as showing: (i) specific coloured probes in the nuclei after the first FISH; (ii) specific coloured probes in the same nucleus after the second FISH and disappearance of signals from the first FISH; and (iii) specific coloured probes in the same nuclei after the third FISH, and disappearance of signals from the second FISH.

**Statistical analysis**

The χ² test was used to compare the results between blastomeres and lymphocytes. P <0.05 was considered to be significant.

**Results**

**Study 1**

A total of 167 blastomeres from 33 embryos were obtained after conventional IVF. Of the 167 blastomeres, 135 had a clearly visible single nucleus in each blastomere. Of these 135 nucleated blastomeres, the nuclei of nine blastomeres disappeared because the membrane of the nucleus was broken during the process of fixation. This problem usually occurred when embryos with poor morphological quality were used. The nuclei of 126 blastomeres were successfully fixed on glass slides and were examined in this study. As shown in Table I, on the basis of the initial number of nuclei used, the rates of nuclear loss and presence of signals were 3 and 92% respectively after the first FISH; 7 and 87% respectively after the second FISH and 13 and 78% respectively after the third FISH.

**Study 2**

The nuclei of 164 lymphocytes were successfully fixed onto glass slides and analysed. As shown in Table I, based on the initial number of nuclei, the rates of nuclear loss and presence of signals were 6 and 91% respectively after the first FISH; 10 and 86%, respectively after the second FISH and 14 and 81% respectively after the third FISH. There was no statistically significant difference in the rates of nuclear loss and present signals between blastomeres and lymphocytes. Probe signals were clearly visible after each FISH procedure (Figure 1).

The FISH results from these 33 embryos are summarized in Table II. Triploid embryos (9/33, 27%) showed all blastomeres to have three sex chromosomes and three chromosomes 11, 13, 18 and 21. Diploid embryos (2/33, 6%) showed all blastomeres to have two sex chromosomes and two chromosomes 11, 13, 18 and 21. Mosaic and chaotic embryos (22/33, 67%) showed blastomeres to have different combinations of chromosomes X, Y, 11, 13, 18 and 21.

**Clinical results**

Oocytes (n = 17) were retrieved from the patient and inseminated with the husband’s spermatozoa. Of the oocytes, 13 out of 17 were 2PN, and nine of 13 fertilized oocytes became 5–8-cell-stage embryos with fair morphological quality. One blastomere was removed from each of the nine embryos. During the process of cell fixation, no nucleus was identified under the microscope in two out of nine biopsied blastomeres (from two embryos at the 5-cell stage at the time of biopsy). These two embryos were cryopreserved without further diagnosis as requested by the patient. Repeated FISH with DNA probes for examination of chromosomes X, Y, 11, 13, 18 and 21 was carried out on seven blastomeres obtained from seven embryos (Table III). Two out of seven blastomeres (from two embryos) revealed a normal complement of chromosomes X, Y, 11, 13, 18 and 21 (both blastomeres displayed signals of one X, one Y, two 11, two 13, two 18 and two 21 chromosomes). These two embryos were transferred into the uterus of the patient on the same day of the PGD. The patient’s human chorionic gonadotrophin (HCG) concentration on 11, 14, 18, 21 and 23 days after transfer of the two normal embryos was 15, 115, 123, 59 and 8 mIU/ml respectively. The results from the other five blastomeres (from five embryos) showed different aneuploidies of chromosomes X, Y, 11, 13, 18 and 21 and are summarized in Table III. These five embryos were cryopreserved in liquid nitrogen as requested by the patient.
Michelman implantation embryos have been discussed previously and the different constitutions of chromosomes in the pre-preimplantation human embryos from 2PN and 3PN oocytes, incidence of chromosomal mosaicism has been observed in of chromosomal mosaicism. After assisted fertilization, a high incidence of nuclei with signals was high after the first of the repeated FISH procedures although the hybridization time was only 30 min. The rate of nuclei with signals decreased in the second and third FISH procedures. The decrease in the efficiency of FISH in the second and third FISH procedures might be due to the repeated washings and denaturation procedures, which may affect the hybridization between DNA probes and target sequences.

The loss of blastomere nuclei may occur in the FISH or repeated FISH procedures, which may be mainly due to embryo quality, poor fixation of the nuclei of blastomeres or the repeated washing procedures. Whether the lack of a signal in some nuclei of the blastomeres is the consequence of the repeated FISH procedures remains to be investigated. From our experience, we believe that the lack of a signal in some blastomeres is mainly related to the quality of embryos and the result of chromosomal abnormalities in blastomeres used in this study. In addition, lack of signals may be also due to chromosomal mosaicism in some embryos. Our results indicate that human embryos from 3PN oocytes have a high incidence of chromosomal mosaicism. After assisted fertilization, a high incidence of chromosomal mosaicism has been observed in preimplantation human embryos from 2PN and 3PN oocytes, and the different constitutions of chromosomes in the pre-implantation embryos have been discussed previously (Michelman et al., 1986; Kola et al., 1987; Munne et al., 1987; Plachot et al., 1987, 1989; Pieters et al., 1992; Jamieson et al., 1994; Kligman et al., 1996; Staessen and Van Steirteghem, 1997).

The loss of lymphocyte nuclei also occurred in the repeated FISH procedures. This may be caused by the repeated washing procedures or by the use of standard glass slides for cell fixation. This problem may be overcome if the glass slides, which have been specially treated to help cells to stick to the surface, are used.

In this study, two DNA probes were used in each of three consecutive FISH procedures. We are currently testing three DNA probes in each of the repeated FISH procedures. The preliminary results were similar to those of the present study (data not shown). So it is possible to analyse six or more chromosomes from a single blastomere using this repeated FISH method. This is an obvious advantage of the repeated FISH procedure over a single FISH procedure. In addition, the initial and repeated FISH procedures can be completed in 6 h allowing embryos to be transferred on the same day the diagnosis is made.

There was no specific reason why Vysis WCP probes were chosen in this study. For convenience, we have always purchased commercial probes for our FISH programme. We did not have our own self-labelled probes. Before the study, we tried probes from two companies, Vysis and Oncor. In our protocol, the probes from Vysis worked better than the probes from Oncor (unpublished observations). We then compared Vysis WCP and centromeric (CEP) probes using interphase nuclei with the same protocol as in the first FISH procedure; both of them gave similar results in terms of FISH efficiency and signal brightness (Liu et al., 1998). We rarely saw fuzzy or diffuse signals in interphase nuclei when WCP probes were used. The major difference between signals from the WCP and CEP probes was that the size of signals of some WCP probes was slightly bigger than that of CEP probes. However, the sizes of two or more signals from a WCP or a CEP probe can be different in an interphase nucleus, especially in the nuclei of blastomeres.

The results also demonstrate that 30 min hybridization time is sufficient when WCP probes are used in interphase nuclei, which is much shorter than that recommended by the manufacturer (4 h to overnight hybridization time is usually recommended by the manufacturer). However, we have no experience with using the WCP probes that are produced from other commercial companies.

Although no ongoing pregnancy was established in our PGD case with this repeated FISH procedure, we were able to detect six chromosomes in single biopsied blastomeres and to transfer embryos into the uterus on the same day of diagnosis. We have no idea why there were more abnormal embryos than normal ones in this patient. The age of the female may be related to these results. Our data were not sufficient to reach any conclusion in this case.

In conclusion, the repeated FISH procedures described here provides the possibility of analysing six or more chromosomes from a single interphase nucleus of an embryo, enabling more information on chromosomes to be obtained from a single cell compared with a single FISH procedure. These procedures are

| Embryos | Stage | No. cells removed | FISH results 1st FISH | 2nd FISH | 3rd FISH | Remarks
|---------|-------|-------------------|-----------------------|---------|---------|--------
| 1       | 7-cell|                  | 2-X, 1-Y              | 2–11, 3–13 | 2–18, 3–21 | Frozen
| 2       | 5-cell|                  | 1-X, 3-Y              | 2–11, 1–13 | 2–18, 2–21 | Frozen
| 3       | 6-cell|                  | 2-X, 3-Y              | 2–11, 4–13 | 2–18, 4–21 | Frozen
| 4       | 8-cell|                  | 2-X, 0-Y              | 2–11, 3–13 | 2–18, 3–21 | Frozen
| 5       | 7-cell|                  | 1-X, 3-Y              | 2–11, 4–13 | 2–18, 1–21 | Frozen
| 6       | 7-cell|                  | 1-X, 1-Y              | 2–11, 2–13 | 2–18, 2–21 | Transferred
| 7       | 7-cell|                  | 1-X, 1-Y              | 2–11, 2–13 | 2–18, 2–21 | Transferred

*The embryos were frozen at the patient’s request.*

Discussion

In order to have more chromosomes examined from an interphase nucleus of a single blastomere, we have developed the repeated FISH procedure. The present study demonstrates that the rate of nuclei with signals was high after the first of the repeated FISH procedures although the hybridization time was only 30 min. The rate of nuclei with signals decreased in the second and third FISH procedures. The decrease in the efficiency of FISH in the second and third FISH procedures might be due to the repeated washings and denaturation procedures, which may affect the hybridization between DNA probes and target sequences.

The loss of blastomere nuclei may occur in the FISH or repeated FISH procedures, which may be mainly due to embryo quality, poor fixation of the nuclei of blastomeres or the repeated washing procedures. Whether the lack of a signal in some nuclei of the blastomeres is the consequence of the repeated FISH procedures remains to be investigated. From our experience, we believe that the lack of a signal in some blastomeres is mainly related to the quality of embryos and the result of chromosomal abnormalities in blastomeres used in this study. In addition, lack of signals may be also due to chromosomal mosaicism in some embryos. Our results indicate that human embryos from 3PN oocytes have a high incidence of chromosomal mosaicism. After assisted fertilization, a high incidence of chromosomal mosaicism has been observed in preimplantation human embryos from 2PN and 3PN oocytes, and the different constitutions of chromosomes in the pre-implantation embryos have been discussed previously (Michelman et al., 1986; Kola et al., 1987; Munne et al., 1987; Plachot et al., 1987, 1989; Pieters et al., 1992; Jamieson et al., 1994; Kligman et al., 1996; Staessen and Van Steirteghem, 1997).

The loss of lymphocyte nuclei also occurred in the repeated FISH procedures. This may be caused by the repeated washing procedures or by the use of standard glass slides for cell fixation. This problem may be overcome if the glass slides, which have been specially treated to help cells to stick to the surface, are used.

In this study, two DNA probes were used in each of three consecutive FISH procedures. We are currently testing three DNA probes in each of the repeated FISH procedures. The preliminary results were similar to those of the present study (data not shown). So it is possible to analyse six or more chromosomes from a single blastomere using this repeated FISH method. This is an obvious advantage of the repeated FISH procedure over a single FISH procedure. In addition, the initial and repeated FISH procedures can be completed in 6 h allowing embryos to be transferred on the same day the diagnosis is made.

There was no specific reason why Vysis WCP probes were chosen in this study. For convenience, we have always purchased commercial probes for our FISH programme. We did not have our own self-labelled probes. Before the study, we tried probes from two companies, Vysis and Oncor. In our protocol, the probes from Vysis worked better than the probes from Oncor (unpublished observations). We then compared Vysis WCP and centromeric (CEP) probes using interphase nuclei with the same protocol as in the first FISH procedure; both of them gave similar results in terms of FISH efficiency and signal brightness (Liu et al., 1998). We rarely saw fuzzy or diffuse signals in interphase nuclei when WCP probes were used. The major difference between signals from the WCP and CEP probes was that the size of signals of some WCP probes was slightly bigger than that of CEP probes. However, the sizes of two or more signals from a WCP or a CEP probe can be different in an interphase nucleus, especially in the nuclei of blastomeres.

The results also demonstrate that 30 min hybridization time is sufficient when WCP probes are used in interphase nuclei, which is much shorter than that recommended by the manufacturer (4 h to overnight hybridization time is usually recommended by the manufacturer). However, we have no experience with using the WCP probes that are produced from other commercial companies.

Although no ongoing pregnancy was established in our PGD case with this repeated FISH procedure, we were able to detect six chromosomes in single biopsied blastomeres and to transfer embryos into the uterus on the same day of diagnosis. We have no idea why there were more abnormal embryos than normal ones in this patient. The age of the female may be related to these results. Our data were not sufficient to reach any conclusion in this case.

In conclusion, the repeated FISH procedures described here provides the possibility of analysing six or more chromosomes from a single interphase nucleus of an embryo, enabling more information on chromosomes to be obtained from a single cell compared with a single FISH procedure. These procedures are
useful for gender determination of an embryo for prevention of X-linked diseases or detection of sex chromosomal and the most common autosomal aneuploidies. The technique has also considerable potential for use in PGD for screening of the most common trisomies in older women who undergo IVF.

References


