Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
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Preimplantation genetic diagnosis for Down syndrome pregnancy*

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Received Aug. 25, 2006; revision accepted Feb. 13, 2007

Abstract: Objective: To evaluate the effect of preimplantation genetic diagnosis (PGD) conducted for women who had Down syndrome pregnancy previously. Methods: Trisomy 21 was diagnosed by using fluorescence in site hybridization (FISH) before embryo transfer in two women who had Down syndrome pregnancies. Each received one or two PGD cycles respectively. Results: Case 1: one PGD cycle was conducted, two oocytes were fertilized and biopsied. One embryo is of trisomy 21 and the other of monosomy 21. No embryo was transferred. Case 2: two PGD cycles were conducted, in total, sixteen oocytes were fertilized and biopsied. Four embryos were tested to be normal, six of trisomy 21, and one of monosomy 21. Five had no signal. Four normal embryos were transferred but no pregnancy resulted. Conclusion: For couples who had pregnancies with Down syndrome previously, PGD can be considered, and has been shown to be an effective strategy.

Key words: Down syndrome, Fluorescence in site hybridization (FISH), Preimplantation genetic diagnosis (PGD)
doi:10.1631/jzus.2007.B0515 **Document code:** A **CLC number:** R715

INTRODUCTION

Down syndrome (DS), also named 21 trisomy syndrome, is a chromosome disorder associated either with an extra chromosome 21 or an effective trisomy for chromosome 21. The population risk for Down syndrome is approximately 1 in 800 live births (Roizen and Patterson, 2003) and the recurrence risk after the birth of an affected child is approximately 1%~2% (Mikkelsen and Stene, 1979; Daniel *et al*., 1982). At present, women who had history of Down syndrome pregnancy may give birth to a normal baby with the help of prenatal diagnosis. However, they may suffer repeated pregnancy terminations following Down syndrome conception and these couples are seeking genetic diagnosis before implantation to avoid subsequent pregnancy termination (Conn *et al*., 1999).

Preimplantation genetic diagnosis (PGD) is a technique that was originally developed as an alternative to prenatal diagnosis for couples at high risk of transmitting a genetic defect. It involves the screening of in vitro fertilization (IVF) or intracytoplasm sperm injection (ICSI) generated embryos by the genetic analysis of one or two biopsied blastomeres, allows scientists to check specific genetic defects of the embryo so that only embryos not affected by the tested disease or balanced for the tested chromosomes will be transferred (Sermon *et al*., 2005). Compared to the traditional methods of prenatal diagnosis, PGD offers genetic analysis at the earliest stage in fetal development, leading to the avoidance of abnormal pregnancy and subsequent pregnancy termination.

Fluorescence in site hybridization (FISH) is the technique of choice for detecting the chromosome status in single cells for PGD, using fluorochrome-labelled DNA probes that are complementary to DNA sequences specific to individual chromosomes. It permits sexing the embryos (in case of X-linked diseases), simultaneous enumeration of up to nine chromosomes for aneuploidy screening (for the detection of abnormal numbers of chromosomes) and structural chromosome abnormalities (such as unbanlanced translocations) (Rubio *et al*., 2005a). FISH has been proved to be a valuable tool for the study of aneuploidy in early human embryos (Schrurs

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* Project supported by the National Basic Research Program of China (Nos. 2006CB944006 and 2006CB504004), the Key Research Program of Zhejiang Province, China (No. 2006C13078) and the Bureau of Science and Technology of Hangzhou, China (No. 20061123B03)

et al., 1993; Munné *et al*., 1998c; 2003). The number of cycles of PGD for aneuploidy screening (PGS) (Sermon *et al*., 2005) has been steadily increasing. However, not many reports of PGD for specific single chromosome 21 were available.

Here, we report two cases whose state of chromosome 21 was detected by using FISH in preimplantation embryos and who had Down syndrome pregnancy. At the same time, the present situation of preimplantation genetic diagnosis used for Down syndrome is discussed.

SUBJECTS AND METHODS

Ethical approval

The clinical application of PGD was licensed by the Health Ministry of People's Republic of China. The protocol was approved by local Ethical Committee and informed written consent was obtained from both patients.

Patients

Case 1: The couple was referred for PGD after three abnormal pregnancies (one spontaneous abortion, a Down syndrome child and one termination of Down syndrome pregnancy). Maternal age was 43 years. Case 2: The couple was referred for PGD after two Down syndrome conceptions (one termination of pregnancy and a Down syndrome child). Maternal age was 30 years. Both couples had normal karyotype as proven by G-banded metaphase chromosome analysis of cultured peripheral blood lymphocytes. Both couples were counseled about the PGD procedure and the need to confirm diagnosis with prenatal diagnosis.

Materials and methods

Ovarian stimulation, fertilization and embryo culture: ICSI was conducted as described by van Steirteghem *et al*.(1993) to avoid sperm contamination. Briefly, patients underwent superovulation and oocyte retrieval was conducted by vaginal ultrasound guided aspiration. Oocytes were subjected to ICSI and assessed for normal fertilization after 17 h. Embryos were cultured in vitro until 44 h post-insemination when the majority had reached the 6~10 cell stage.

Biopsy and slide preparation: On day 3 the embryos biopsy procedure was performed after decompaction in Ca/Mg-free Scandinavian Embryo Medium (Science Scandinavia) and acidified Tyrode's solution for zona drilling. One blastomere was biopsied from each embryo. After biopsy, embryos were immediately returned to normal culture conditions. Each single blastomere was transferred to poly-L-lysine slides in spreading solution (0.01 mol/L HCl, 0.1% Tween 20) that was gently agitated until lysis occurred and all nuclei were clear of cytoplasm. The slides were left to air dry for ~20 min, washed in PBS for 5 min and dehydrated through an ethanol series (70%, 80%, 100% at 5 min each).

FISH: FISH was conducted as Munné *et al*.(1998a) described. Biopsied blastomeres were hybridized with probe of locus-specific indicator (LSI) 21 (SpectrumOrange) provide by Vysis Inc. (Illinois, USA) and LSI (13q34) (SpectrumGreen) was used as control. FISH signals were visualized using an Olympus-AX fluorescence microscope. Only embryos with normal chromosomal complement were transferred on day 4 of culture while those exhibiting chromosomal abnormalities were excluded.

RESULTS

Case 1

One PGD cycle was conducted. Three oocytes were obtained, of which two were fertilized normally and biopsied on day 3. FISH results showed that one is of trisomy 21, and the other is of monosomy 21. No embryo was transferred (Figs.1a and 1b).

Case 2

Two PGD cycles were conducted. In the first cycle, ten oocytes were obtained, of which eight were fertilized normally and biopsied on day 3. Two gave a normal/balanced signal pattern, and were transferred on day 4. Three embryos were proved to be trisomy 21, and one of monosomy 21. The other two embryos were inconclusive because of hybridization failure. No pregnancy resulted. In the second cycle, seventeen oocytes were obtained, fourteen were fertilized normally, and eight biopsied on day 3. Two gave a normal/balanced signal pattern, and were transferred on day 4. Three embryos were proved to be trisomy 21. The other three embryos were inconclusive. No pregnancy resulted (Figs.1c and 1d).

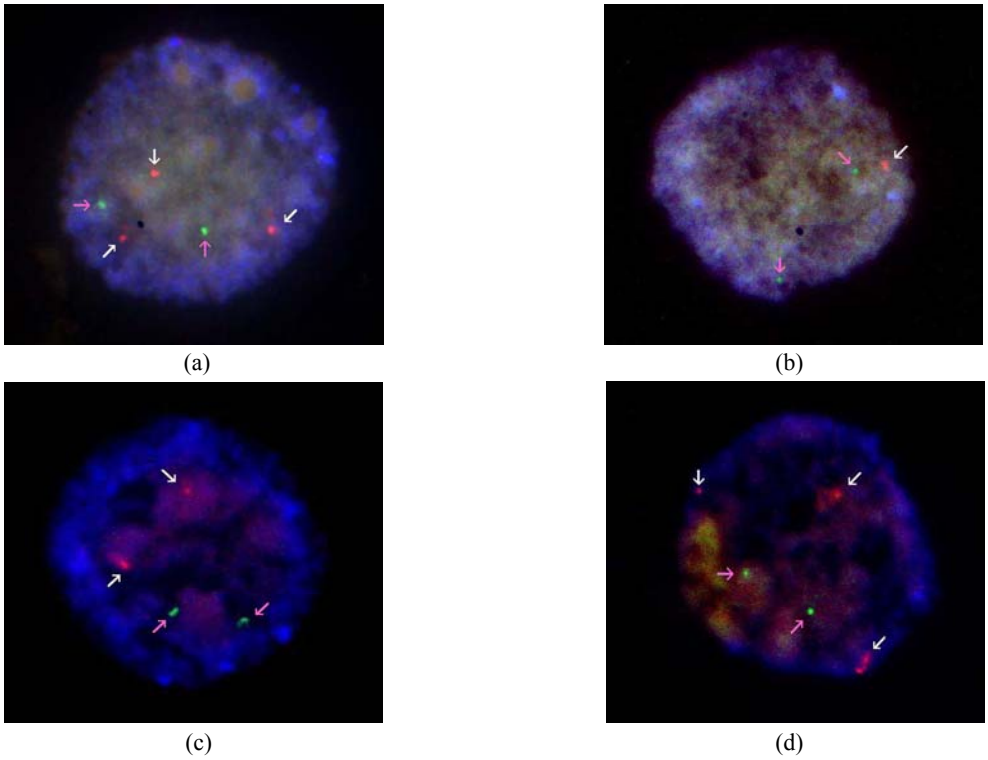

Fig.1 Results of FISH analysis of biopsied blastomeres from preimplantation embryos using chromosome 21 (orange, white arrow) and chromosome 13 (green, pink arrow) probes. Case 1: (a) trisomy 21 (three orange signals), (b) monosomy 21 (one orange signal); Case 2: (c) normal for chromosome 21 (two orange signals), (d) trisomy 21 (three orange signals). All of the blastomeres are normal for chromosome 13 (two green signals)

DISCUSSION

Down syndrome is classically characterized by birth defects and mental retardation. In conventional prenatal diagnosis, definitive diagnosis of Down syndrome requires amniocentesis, chorionic villus sampling or umbilical blood sampling. Efforts have been made to reduce the risk of prenatal diagnosis, including obtaining fetal cells from maternal blood (Ho *et al*., 2003; Yang *et al*., 2003; 2006), applying molecular genetic techniques to broaden the range of identifiable genetic disorders (Solassol *et al*., 2003; Yang *et al*., 2006) and raising the detection rate of noninvasive prenatal screening to reduce the false-positive rate in the hope of reducing the examine rate of invasive procedure (Cicero *et al*., 2003; Muller *et al*., 2003; Nicolaides, 2004; Spencer *et al*., 2003). However, invasive diagnosis is unavoidably combined with some complications and there are many religious controversies over whether to terminate the affected pregnancy. Moreover, the termination of pregnancy in the event of Down syndrome is not acceptable for some couples and there is growing demand for PGD diagnosis of Down syndrome.

PGD is an alternative to prenatal diagnosis combined with assisted reproductive technology (ART) and genetic diagnosis technology, which offers genetic analysis at the earliest stage in fetal development, allows unaffected embryos to be identified and transferred to the uterus (Munné *et al*., 2004). PGD avoids not only pregnancy termination when the fetus is proved affected by prenatal diagnosis, but also potential complications of invasive procedures, such as abortion, hemorrhage and uterus infection. Moreover, PGD can solve the political and ethical controversy of whether to terminate the affected pregnancy. Since Handyside *et al*.(1990) reported the first established pregnancy using PGD in 1990, the PGD cycles have been stably increasing worldwide (Geraedts *et al*., 2000; Harper *et al*., 2006; Sermon *et al*., 2005), and PGD has been extensively applied in sex-linked disorders, single gene disease, autosomal abnormalities, and aneuploid detection of women with advanced age. Therefore, PGD has been a practical

option for avoiding the birth of affected children, representing an important complement to traditional prenatal diagnosis (Kuliev and Verlinsky, 2004).

PGS was applied primarily to infertile women with the following indications: advanced maternal age (AMA) (Kahraman *et al*., 2000), recurrent implantation failure (RIF) (Caglar *et al*., 2005; Taranissi *et al*., 2005) and recurrent miscarriage (RM) (Rubio *et al*., 2005b). PGD for numerical chromosome abnormalities has four potential benefits: (1) to prevent trisomic offspring by analyzing chromosomes 13, 16, 18, 21 and 22 (Kuliev *et al*., 2003); (2) to reduce spontaneous abortions (Munné *et al*., 1998b; Rubio *et al*., 2003); (3) to reduce multiple pregnancy by minimizing the number of embryos necessary for replacement and successful pregnancy (Munné, 2002); and (4) to improve implantation. Many cycles of PGS were performed worldwide using FISH probes for chromosome 13, 18, 21 and others. However, only 4 reports of 5 cases of PGD for specific chromosome 21 are available (Conn *et al*., 1998; 1999; Scriven *et al*., 2001; Luo *et al*., 2002). All cases had history of Down syndrome pregnancy. PGD was conducted using cleavage stage embryo biopsy and FISH analysis for chromosome 21. Each couple underwent one or two treatment cycles. The case information and results are summarized in Table 1.

Table 1 The case information and PGD results

Reference	Case	Maternal age	Pregnancy times (DS pregnancies)	Parental karyotypes	Cycle	Oocytes			Biopsy diagnosis	Transferred	Outcome
						Collected	Fertilized	Biopsied			
Conn *et al*. (1998)	1[a]	31	4 (3)	45, XX, der (13; 21) (q10; q10)	1	11	8	6	2 normal	Yes	NP
									2 monosomy 21, 13	No	
									1 monosomy 21	No	
									1 monosomy 13	No	
					2	14	10	5	2 monosomy 21, 13	No	
									1 tetrasomy 21	No	
									1 trisomy 21	No	
									1 unconclusive	No	
Conn *et al*. (1999)	2	36	4 (3)	Normal	1	31 (total)	13 (total)	2	2 trisomy 21	No	
					2			3	2 normal	Yes	NP
									1 trisomy 21	No	
	3	32	5 (1)	46, XX, t (6; 21) (q13; 22.3)	1	32 (total)	11 (total)	4	1 normal	Yes	ABP
									2 trisomy 21	No	
									1 monosomy 21	No	
					2			0			
Scriven *et al*.(2001)	4[b]	39	2 (1)	45, XX, der (14; 21) (q10; q10)	1	11	7	5	3 normal	Yes	NP
									1 normal	No	
									1 trisomy 21, 14	No	
					2	15	10	9	3 normal	Yes	OHC
									1 trisomy 21	No	
									1 monosomy 21	No	
									1 trisomy 14	No	
									1 monosomy 14	No	
									2 unconclusive	No	
Luo *et al*. (2002)	5	30	1 (1)	Normal	1	14	13	8	3 normal	Yes	OHC
									3 normal	No	
									1 trisomy 21	No	
									1 no signal	No	
Author's cases	6 (case 1)	43	3 (2)	Normal	1	3	2	2	1 trisomy 21	No	
									1 monosomy 21	No	
	7 (case 2)	30	2 (2)	Normal	1	10	8	8	2 normal	Yes	NP
									3 trisomy 21	No	
									1 monosomy 21	No	
									2 unconclusive	No	
					2	17	14	8	2 normal	Yes	NP
									3 trisomy 21	No	
									3 unconclusive	No	

[a]Probes: Locus-specific indicator (LSI) 13 and 21; [b]Probes: LSI 21 and a biotinylated 14q subtelomere probe. NP: No pregnancy; OHC: One healthy child; ABP: A biochemical pregnancy

The PGD cycles summarized here were conducted for women who had pregnancies of Down syndrome previously. In total of above summarized reports and ours, 60 embryos were biopsied. Of them, only 22 (36.7%) were tested to be normal for chromosome 21, 18 (30.0%) transferred, 2 clinical pregnancies resulted, and two healthy children were born and no Down syndrome pregnancy occurred. In addition, the FISH results of surplus embryos (not biopsied because of fewer than six cells on day 3) showed that the abnormality rate was as high as 81.8% (18/22) (Conn *et al*., 1998; 1999; Cozzi *et al*., 1999; Scriven *et al*., 2001; Luo *et al*., 2002). These data indicate that applying PGD for couples with history of Down syndrome effectively avoided affected pregnancy, although the pregnancy rate is low. The data here also imply that successful pregnancy has more possibility occurring in couples with high rate of normal embryos (50% and more), as suggested by Scriven *et al*.(2001), Conn *et al*.(1998; 1999) and Luo *et al*.(2002).

Women who have a history of Down syndrome pregnancy are at high risk of recurrent Down syndrome (Hook, 1992). Data from livebirths and from amniocenteses in Europe (Stene *et al*., 1984; Warburton *et al*., 1987) showed that for women with a history of trisomy 21, there is an increased recurrence risk of trisomy 21, but not other trisomy. Although a wider selection of commercial probes is now becoming available, the development of suitable probe combinations for detecting multiple chromosome abnormality is expensive (Conn *et al*., 1999). Therefore, for patients with a history of Down syndrome pregnancy, applying PGD for chromosome 21 is practicable (Scriven *et al*., 2001; Luo *et al*., 2002).

The cause of recurrent Down syndrome is complex. Although studies have shown that most of recurrent trisomy 21 pregnancies may be the result of chance alone (Panaqalos *et al*., 1992), the possibility of parental translocation and gonadal mosaicism has important implications for recurrence risk. Other evidence suggests that women with a diminished ovarian reserve may have a higher risk of a trisomic conception, so that biological ovarian age may be a better indicator of trisomy risk than chronological age (van Montfrans *et al*., 2001). Recently, many suggestions for the mechanisms of non-age-related risks (absolute excess risk) have been put forward, indicating that women who experienced a Down syndrome pregnancy at a young age have a greater absolute excess risk of recurrence than those whose first Down syndrome pregnancy was at an older age (Morris *et al*., 2005). Thus, the causative reasons for high recurrence of Down syndrome in our cases and in Luo *et al*.(2002)'s in which both parents had a normal chromosome complement are needed for further analysis.

In conclusion, PGD for trisomy 21 with FISH avoids affected pregnancy and offers favorable results. Although standard prenatal diagnosis remains the method of choice for aneuploidy, for couples who had pregnancies with Down syndrome previously, PGD can also be considered, and has been shown to be an effective strategy.

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