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ARTICLE

First successful pregnancy following PGD for chromosome translocation on embryos generated from in-vitro matured oocytes: a case report

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Asangla Ao received her PhD in genetics and biometry from University College London, UK and her postdoctoral training in the USA. She then worked as a senior research scientist in Royal Postgraduate Medical School at Hammersmith Hospital, London, UK on preimplantation genetic diagnosis (PGD). At present, she is an associate professor in the Department of Obstetrics and Gynecology and Department of Human Genetics and the scientific director of the PGD programme at McGill Reproductive Center. Her research interests include many aspects of human embryology, such as chromosome abnormality in human embryos, gene expression in mammalian preimplantation embryos and PGD.

Abstract As far as is known, this is the first report of a successful pregnancy outcome following preimplantation genetic diagnosis for a chromosome translocation in embryos generated from in-vitro matured oocytes. A couple presented to the study clinic where the female partner was a carrier of the reciprocal chromosome translocation 46,XX,t(1;20)(p36.1;p12.2) with three consecutive pregnancy terminations due to either fetal abnormality or unbalanced translocation products detected in the conceptus. Under routine ultrasound investigation she was diagnosed with polycystic ovaries. The patient underwent an in-vitro maturation/preimplantation genetic diagnosis cycle where the immature oocytes were matured *in vitro* and fertilized by intracytoplasmic sperm injection. Day-3 embryos were screened for the chromosome abnormality by fluorescent in-situ hybridization. A single embryo diagnosed as chromosomally normal/balanced was transferred on day 5 and resulted in the birth of a healthy child.

KEYWORDS: chromosome translocation, FISH, in-vitro maturation, polycystic ovarian syndrome, preimplantation genetic diagnosis

Introduction

Preimplantation genetic diagnosis (PGD) is now available for couples carrying inherited genetic diseases such as single gene defects or balanced chromosome translocations to choose unaffected embryos for transfer (Basille et al., 2009; Munne, 2005; Munne et al., 2009; Scriven et al., 1998; Spits and Sermon, 2009; Verlinsky et al., 2004). To undergo the PGD procedure, the couple have to undergo IVF to obtain a sufficient number of embryos for genetic analysis so that there are some unaffected embryos available for transfer. The IVF treatment involves ovarian stimulation with fertility

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medications to induce multiple follicular growth before oocyte retrieval. Patients with polycystic ovaries or polycystic ovarian syndrome have a higher risk of developing ovarian hyperstimulation syndrome following hormonal treatment used for the conventional IVF procedure (Delvigne et al., 1993; MacDougall et al., 1993; Tummon et al., 2005). Recent advances in in-vitro maturation (IVM) to treat women with polycystic ovarian syndrome-related infertility have been successful and good pregnancy rates have been achieved (Chian et al., 1999). Unlike the conventional IVF procedure where mature oocytes are used, in the IVM procedure immature oocytes recovered from unstimulated patients are matured *in vitro* before fertilization. Although pregnancy rates are lower than those obtained in conventional IVF, recent reports show an improvement in the pregnancy rate in IVM cycles following human chorionic gonadotrophin (HCG) priming (Chian et al., 1999, 2000; Son et al., 2006, 2008).

Balanced translocations are more prevalent among infertile couples and patients with a history of recurrent pregnancy loss (Tharapel et al., 1985). Several studies have shown that PGD can be an alternative for carriers of balanced translocations to reduce the risk of repeated pregnancy loss and avoid the risk of termination of an affected pregnancy (Fischer et al., 2010; Munne et al., 2000; Otani et al., 2006). The success rate following PGD for translocation depends on the number of unaffected embryos with good morphology available for transfer. The present study was designed to investigate whether a sufficient number of oocytes could be retrieved from a patient with polycystic ovaries who was also a carrier of a chromosome translocation to perform PGD on embryos generated through IVM of oocytes.

Materials and methods

Patient details

In June 2006, a couple (31-year-old female and 34-year-old male) was referred to the fertility clinic. The couple had an uneventful first pregnancy resulting in the birth of a healthy baby girl. The next pregnancy was terminated at 16 weeks due to fetal abnormality. Karyotype analysis of the couple showed that the female partner was a carrier of a balanced reciprocal translocation between the short arms of chromosomes 1 and 20, 46,XX,t(1;20)(p36.1;p12.2). The next two consecutive pregnancies were terminated following chorionic villus sampling due to the presence of unbalanced chromosome complements. The prenatal diagnosis results were 46,XX,der(1)t(1;20)(p36.1;p12.2)mat and 46,XY,der(1)t(1;20)(p36.1;p12.2)mat.

The female patient was diagnosed with polycystic ovaries under routine ultrasound investigation. Semen analysis for the male partner was normal. The couple was offered IVF or IVM followed by PGD for translocation. The couple was counselled regarding these two procedures and they opted to undergo IVM followed by PGD. The initial PGD and IVM studies were approved by the research ethics board of the McGill University Health Centre, at the Royal Victoria Hospital.

In-vitro maturation and embryo biopsy procedure

To initiate the treatment cycle, the 31-year-old patient was monitored regularly by ultrasound scan in a normal menstrual cycle. When the largest follicle reached 10—12 mm in diameter, a dose of 10,000 IU HCG was administered and 35—38 hours later transvaginal ultrasound-guided oocyte retrieval was performed using a 19-gauge single lumen aspiration needle (K-OPS-7035-RWH-ET; Cook, Australia) at an aspiration pressure of 7.5 kPa. The oocyte maturity was assessed after oocyte collection. If no germinal vesicle (GV) was observed in the oocyte cytoplasm, the cumulus masses were removed and the oocyte maturity was reassessed. The immature oocytes at the GV or GV-breakdown (GVBD) stages were cultured in IVM medium (Cooper Surgical, CT, USA) supplemented with 75 mIU/ml FSH and LH. Following culture on day 1 (24—30 h), the cumulus masses were removed and oocyte maturity was examined. After the examination, immature oocytes were further cultured in the same medium and the maturity was reexamined after 36 and 48 h of culture. Matured oocytes were inseminated by intracytoplasmic sperm injection (ICSI) using her partner's spermatozoa. Fertilization was examined 17—19 h after ICSI for the presence and number of pronuclei. Embryos having two pronuclei were cultured in cleavage medium (Cook, Canada).

For the preparation of the endometrium, the patient was given 6 mg oestradiol (Estrace; Roberts Pharmaceutical, Mississauga, Ontario, Canada) in divided doses starting on the day of oocyte retrieval. Luteal support was provided in the form of intravaginal progesterone (Prometrium; Schering, Pointe-Claire, Quebec, Canada) at a dose of 400 mg three times daily for 12 weeks starting on the day of ICSI.

Biopsy of a single blastomere was performed on day-3 post insemination as described previously (Lorusso et al., 2006). Briefly, each embryo was placed in a droplet containing Ca^{2+} and Mg^{2+}-free medium (Cook Canada, Stouffville, ON, Canada) and a hole was drilled in the zona pellucida using 1.48 μm infrared diode laser in computer-controlled non-contact mode (Hamilton Thorn Inc., MA, USA). A single blastomere was then aspirated gently through the hole. The biopsied embryo was returned to culture medium while the biopsied blastomere was washed through three drops of phosphate-buffered saline (PBS) containing 4 mg/ml bovine serum albumin (Sigma, Oakville, ON, Canada), then it was transferred onto a poly-L-lysine coated glass slide and fixed with spreading buffer (0.01 mol/l HCl/0.1% Tween 20) under an inverted microscope. The number of fixed nuclei for each blastomere was recorded; the slides were air-dried, washed in PBS and dehydrated with an ethanol series for 2 min each.

Chromosome analysis was performed using fluorescence in-situ hybridization (FISH) with commercially available probes. TelVysion 1p (Locus: CEB108/T7, spectrum green) and CEP 20 (Locus: Alpha Satellite DNA, spectrum orange) were used in the first round of hybridization followed by TelVysion 20p (Locus: 20p18, D20S1157, spectrum green) and CEP 1 (Locus: Satellite III DNA, spectrum orange; Vysis, Downers Grove, IL, USA) were used in the second round. Preclinical experiments were carried out using these FISH probes in interphase and metaphase lymphocytes obtained from the couple's blood. In addition, nuclei from donated spare embryos were used to test the hybridization efficiency of these probes. Briefly, the nuclei and probe mixture were co-denatured at 75°C for 5 min and then hybridized in a moist chamber overnight at 37°C for both rounds of

hybridization. Unbound probes were removed with 0.4× standard saline citrate (SSC)/0.3% Tween 20 with stringent washing at 70°C for 2 min; the slides were then rinsed in 2× SSC/0.1% Tween 20 at room temperature. The preparations were mounted in antifade solution (*p*-phenylenediamide dihydrochloride; Vector, Burlingame, CA) containing 0.25 ng/ml 4′,6-diamidino-2-phenylindole (Sigma) nuclear stain. The signals were observed under a fluorescence microscope (Olympus CK60; Olympus Canada, Inc.) with the appropriate filter sets. The embryos diagnosed as balanced or normal were transferred to the patient on day 5 post insemination.

Results

In total 20 immature GV- or GVBD-stage oocytes were retrieved, 10 from each ovary. A total of 14 immature oocytes reached metaphase II (MII) stage, which included 10 oocytes matured after 24 h of in-vitro culture, two oocytes reached the MII stage after 36 h of culture and two after 48 h of culture (**Table 1**). The remaining six oocytes did not progress beyond the GV stage and were discarded. All 14 MII oocytes were normally fertilized following ICSI and all embryos were biopsied on day 3. All 14 embryos yielded results following the FISH procedure. Out of the 14 embryos analysed, only one embryo was diagnosed as chromosomally normal/balanced for the chromosomes tested. The remaining 13 embryos were diagnosed as abnormal (**Table 1**). The single embryo diagnosed as normal/balanced was transferred on day 5 at the compacted morula stage and resulted in the birth of a healthy child at 39 weeks of pregnancy. As far as is known, the present case is the first successful pregnancy following IVM/PGD for chromosome translocation.

Discussion

Couples who are carriers of chromosome translocations are generally counselled for the expected rate of unbalanced offspring based on their karyotype. However, based on their karyotype, neither the chances of a normal pregnancy nor the rate of miscarriage can be correctly predicted. Therefore, for patients undergoing PGD for translocation, the number of transferable and non-transferable embryos can only be obtained after genetic analysis of their embryos.

For any type of PGD cycle to be successful, it is important that suitable numbers of embryos are biopsied so that there are ideally two or more unaffected good-quality embryos available for transfer. Usually the translocation patient undergoes ovarian stimulation cycles to obtain a sufficient number of embryos for the PGD treatment. The present couple was offered an IVM cycle where immature eggs were matured *in vitro* before fertilization followed by embryo biopsy for PGD. The couple was counselled as one of the possible limitations for an IVM cycle could be availability of fewer embryos for PGD. In this present case, the number of embryos generated *in vitro* was not the limiting factor; 14 embryos were obtained that could be biopsied,

Table 1 Embryological and preimplantation genetic diagnosis data.

Variable	*Maturation stage*	*Fluorescence in-situ hybridization analysis*	
		Day-3 embryos	*Spare embryos (reanalysis)*
Oocytes retrieved	10 MI	—	—
	10 GV	—	—
Oocytes matured *in vitro*	10 MII at 24 h	—	—
	2 MII at 36 h	—	—
	2 MII at 48 h	—	—
	6 GV at 48 h (discarded)	—	—
Fertilized oocytes after ICSI	14	—	—
Embryos biopsied	14	1 transferable	1 haploid or 4:0 segregation
		13 non-transferable	3 adjacent-1
		2 adjacent-1	3 3:1
		1 adjacent-2	2 no SP
		3 3:1	4 no analysis[a]
		1 haploid or 4:0 segregation	
		1 triploid or 4:0 segregation	
		5 no SP	

GV = germinal vesicle; ICSI = intracytoplasmic sperm injection; MI = metaphase I; MII = metaphase II; SP = segregation pattern.
[a]Degenerated embryos.

a number comparable to that obtained in a regular IVF cycle. There was, however, only one embryo that was diagnosed as normal or balanced and the rest of them were diagnosed as abnormal (**Table 1**). Nine of the non-transferred embryos were reanalysed and three distinct segregation patterns were observed: three consistent with adjacent-1 segregation, three consistent with 3:1 segregation and one haploid or 4:0 segregation. No segregation pattern was observed in the remaining of the two embryos. These results were in agreement with those obtained on day 3 when blastomeres from the same embryos were analysed except that one of the reanalysed embryos with adjacent-1 segregation pattern had been previously diagnosed as having no segregation pattern on day 3. In addition, one of the two reanalysed embryos with no segregation pattern had been diagnosed previously with triploidy or 4:0 segregation pattern on day 3. The adjacent-1 segregation pattern detected in three of the reanalysed embryos was also seen in two chorionic villus samples following prenatal diagnosis before the patient opted for PGD.

The remaining four embryos had degenerated and therefore no further analysis could be carried out. The chromosome analysis was performed using only two chromosome probes involved in the translocation therefore the presence of aneuploidy, if any, could not be identified. It is also conceivable that one embryo diagnosed as haploid or 4:0 segregation and the second one diagnosed as triploid or 4:0 segregation could be possible double aneuploidy (double monosomy and double trisomy) as cleavage-stage embryos with similar chromosome complements have been reported (Ao et al., 2006; Benkhalifa et al., 2009; Bielanska et al., 2002; Goossens et al., 2008; Magli et al., 2007; Mantzouratou et al., 2007; Munne et al., 2007; Requena et al., 2009; Zhang et al., 2010). Unlike the information available on chromosome abnormality rate in embryos obtained from IVF treatment, the incidence of chromosome abnormality in IVM embryos has been reported in only a few small studies (Ao et al., 2006; Benkhalifa et al., 2009; Requena et al., 2009; Zhang et al., 2010). Requena et al. (2009) reported preimplantation genetic screening (PGS) in 20 IVM embryos obtained from 11 patients undergoing egg donor cycles. The control group in their study was 200 embryos from 25 patients undergoing PGD for sex selection of inherited X-linked diseases by FISH. The difference in the aneuploidy rates between these two groups was not statistically significant. In a recent report (Zhang et al., 2010), the chromosome abnormality rate was compared between IVM and IVF embryos obtained from six and 30 PGS cycles, respectively. Similar to the study reported by Requena and colleagues (2009), the rates of chromosome abnormality were not significantly different between these two groups of patients. However, the detailed analysis of spare embryos showed a higher rate of chaotic embryos in the IVM group compared with the IVF group. In another study, Benkhalifa et al. (2009) analysed 188 arrested IVM embryos at the cleavage stage and reported a lower incidence of chromosome abnormality compared with the other two studies. It is, therefore, difficult to draw a definitive conclusion on the incidence of chromosome abnormalities in IVM embryos. There is, however, no clear evidence suggesting that the IVM embryos may have a higher rate of aneuploidy compared with those generated by the standard IVF procedure.

The success of the present case study shows that the number of matured eggs and, ultimately, total number of cleavage-stage embryos generated from in-vitro matured oocytes were comparable to a standard IVF cycle. IVM/PGD may be offered to patients with polycystic ovaries or polycystic ovarian syndrome for other genetic diseases.

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