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Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of β-thalassaemia: a pilot study

G.Kokkali[1,2,6], J.Traeger-Synodinos[2], C.Vrettou[2,3], D.Stavrou[1], G.M.Jones[4], D.S.Cram[4,5], E.Makrakis[1], A.O.Trounson[4,5], E.Kanavakis[2] and K.Pantos[1]

[1]Centre for Human Reproduction, Genesis Hospital, Athens, Greece, [2]Laboratory of Medical Genetics, Athens University, [3]Research Institute for the Study of Genetic and Malignant Disorders in Childhood, St Sophia's Children's Hospital, Athens, Greece, [4]Monash Immunology and Stem Cell Laboratories, Monash University, Melbourne, Australia and [5]Monash IVF, Melbourne, Australia

[6]To whom correspondence should be addressed at: Center for Human Reproduction, Genesis Hospital, Papanikoli Avenue 14-16, Halandri, Athens 152-32, Greece. E-mail: georgiakokkali@mail.com

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BACKGROUND: Trophectoderm biopsy at the blastocyst stage is an emerging approach in preimplantation genetic diagnosis (PGD). This study aimed to compare genotyping success and implantation rates in PGD cycles for β-thalassaemia following biopsy at the cleavage versus the blastocyst stage, with transfer of blastocysts. METHODS: This pilot study included 20 cycles: Group A: 10 cycles, day 3 blastomere biopsy, day 5 transfer; Group B: 10 cycles, day 5 trophectoderm biopsy, day 6 transfer. Standard-assisted reproduction and laser biopsy procedures were used. Biopsied cells were genotyped using real-time PCR multiplexed with fluorescent microsatellite analysis. RESULTS: In Group A, 131 fertilized eggs developed to 101 embryos suitable for single blastomere biopsy; 76/101 blastomeres were diagnosed (75.2%), 30 unaffected blastocysts were transferred resulting in six pregnancies (eight fetal hearts, 26.7% implantation rate). In Group B, 128 fertilized eggs developed to 53 blastocysts for trophectoderm biopsy (four to five cells), with 50/53 blastocysts diagnosed (94.3%), 21 unaffected blastocysts transferred and 6 pregnancies initiated (10 fetal hearts, 47.6% implantation rate). Overall, nine pregnancies reached >10 weeks gestation and were confirmed unaffected by prenatal diagnosis, with 12 healthy babies born. CONCLUSIONS: This pilot study suggests that trophectoderm biopsy and blastocyst transfer may be more advantageous than cleavage stage biopsy with respect to outcome of PGD for monogenic diseases.

Key words: biopsy/blastocyst/laser/preimplantation genetic diagnosis/β-thalassaemia

Introduction

Preimplantation genetic diagnosis (PGD) and single cell genetic analysis has become increasingly more popular since its first application (Handyside *et al.*, 1990) as an alternative to prenatal diagnosis to avoid termination of pregnancy in couples at high risk of transmitting a genetic defect. There are three potential sources of embryonic genetic material for preimplantation genetic analysis: polar bodies (biopsy of the first polar body from oocytes before sperm insemination or biopsy of both polar bodies from oocytes after sperm insemination), blastomeres from cleavage stage embryos and trophectoderm cells from embryos at the blastocyst stage.

In clinical practice, polar body biopsy has been used primarily for aneuploidy screening (Verlinsky *et al.*, 1996) and less commonly for the detection of maternal transmission of single gene defects (Verlinsky *et al.*, 1997) such as β-thalassaemia (Kuliev *et al.*, 1998). According to the ESHRE PGD Consortium data collections, cleavage stage biopsy, usually involving aspiration of one to two blastomeres, is the most common approach for attaining embryonic genetic material for PGD analysis (Harper *et al.*, 2006). Precompaction eight-cell embryos are usually biopsied early on day 3 (insemination on day 0), and following genetic diagnosis, the embryo transfer may be performed on the same day (Boada *et al.*, 1998) or delayed to day 4 or until the embryo has reached the blastocyst stage.

Since the application of extended culture using more complex media or new generation sequential media, high rates of development to the blastocyst stage have been reported (36–66%), regardless of whether all the embryos or only those surplus to the needs of transfer were cultured to the blastocyst stage (Muggleton-Harris *et al.*, 1995; Scholtes and Zeilmaker, 1996; Desai *et al.*, 1997; Jones *et al.*, 1998a,b; Jones and Trounson, 1999; Gardner and Lane, 1998; Gardner *et al.*,

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1998a,b; Rijnders and Jansen, 1998). Several reports have indicated that pregnancy rates following blastocyst stage transfer are improved compared to transfer at earlier stages (Jones *et al.*, 1998a,b; Gardner *et al.*, 1998a,b; Rijnders and Jansen, 1998; Schoolcraft *et al.*, 1999; Pantos *et al.*, 2001) and pregnancies have resulted after day 3 biopsy and embryo transfer on day 4, 5 or 6 (Grifo *et al.*, 1998; Palmer *et al.*, 2002).

The main disadvantage of PGD based on analysis of polar body or blastomere biopsy procedures is the limited amount of material available for genetic analysis. When diagnosing monogenic disorders in single cells using PCR-based protocols, there is a high risk of PCR failure (no result) and allele dropout (ADO) (incomplete result), potentially resulting in a reduced number of unaffected embryos available for transfer. Increasing the amount of starting DNA template should in principle increase the sensitivity and reliability of genetic diagnosis. Hence, the biopsy of multiple trophectoderm cells from the blastocyst rather than a single cell from cleavage stage embryos should potentially lead to improved PGD outcome for patients. Blastocyst trophectoderm biopsy using micromanipulation methods was first reported by Dorkas *et al.* (1990), although not in the context of clinical application. The development of noncontact lasers has greatly facilitated trophectoderm biopsy, first to make a hole in the zona and secondly, following a period in culture, to excise trophectoderm cells (Veiga *et al.*, 1997). Pregnancies following blastocyst biopsy and PGD have recently been reported (de Boer *et al.*, 2004; Kokkali *et al.*, 2005).

The purpose of the present study was to evaluate and compare the diagnosis and implantation success rates between patients undergoing blastomere biopsy and blastocyst transfer and those having trophectoderm biopsy and blastocyst transfer for the diagnosis of β-thalassaemia.

Materials and methods

Patient recruitment and treatment

The study included 20 couples in whom both partners were β-thalassaemia carriers who requested PGD to avoid initiation of a pregnancy affected with homozygous β-thalassaemia. All cycles were performed between January 2004 and May 2005 in collaboration between the Centre for Human Reproduction, Genesis Hospital, Athens, and the Laboratory of Medical Genetics, Athens University, Greece. All couples underwent an initial consultation at both the Centre for Human Reproduction and the Laboratory of Medical Genetics to acquire information on all aspects of the IVF and PGD procedures and to give written informed consents. All the couples underwent the basic fertility work-up (clinical examination, semen analysis, hysterosalpingogram, transvaginal ultrasound scan, cycle day-2 female hormonal profile) at the Centre for Human Reproduction. They also provided blood samples for DNA analysis to determine their β-globin gene mutations (Vrettou *et al.*, 2003).

All the study couples underwent controlled ovarian stimulation with a long protocol (down-regulation with GnRH agonist and stimulation with recombinant FSH) (Pantos *et al.*, 1994), transvaginal oocyte retrieval and fertilization following ICSI as previously described (Yanagimachi, 2005). Only the first cycle for each couple was included in the study. Study subjects were randomly allocated (randomization in blocks with the use of random number tables) to two groups according to the day of embryo biopsy: Group A included couples receiving day 3 biopsy and Group B included couples receiving day 5 biopsy.

Embryo culture

All embryos were cultured in vitro in 10 μl microdrops of G1:3 culture medium (Vitrolife Goteborg, Sweden), and on day 3 post-fertilization, they were transferred to 20 μl microdrops of G2:3 (Vitrolife Goteborg, Sweden) for culture to the blastocyst stage (Jones *et al.*, 1998a) under standard incubation conditions. All embryo transfers were performed at the blastocyst stage.

Cleavage stage biopsy (Group A)

In Group A, embryos that had six or more cells early on day 3 after fertilization were placed into 20 μl of G-MOPS medium (Vitrolife Goteborg, Sweden) under mineral oil and subjected to biopsy following zona ablation using a noncontact laser (ZILOS-tk; Hamilton Thorne Biosciences, Beverley, MA, USA). One blastomere from each embryo was removed using micromanipulation and placed immediately in RNAse–DNAse-free PCR tubes containing 10 μl of PCR-grade water. Following the addition of Proteinase K (final concentration 50 μg/ml; Roche Molecular Biochemicals, Mannheim, Germany), the samples were overlaid with oil and transferred to the Laboratory of Medical Genetics for DNA diagnosis (Traeger-Synodinos *et al.*, 2003; Vrettou *et al.*, 2004). Following the biopsy, embryos were further cultured until day 5 after fertilization and unaffected blastocysts were transferred to the patient's uterus using a Wallace catheter (SIMS Portex Ltd, Hythe, UK). On day 10 after embryo transfer, a serum β-HCG test was performed to evaluate initiation of a pregnancy, and at 6 weeks of gestation, the pregnancy was confirmed by ultrasound detection of fetal heart beat.

Blastocyst biopsy (Group B)

In Group B, embryos were cultured to the blastocyst stage, and on day 5 after fertilization, they were assessed for blastocyst formation. All embryos that developed to the blastocyst stage (early to expanded stage) had four to five trophectoderm cells biopsied in two steps (Kokkali *et al.*, 2005). At about 116 h after fertilization (morning of day 5), a hole was made in the zona pellucida directly opposite the inner cell mass of each blastocyst, using the lowest setting of the ZILOS-tk noncontact laser (Hamilton Thorne Biosciences). Blastocysts were incubated for a further 4 h to allow blastocoele expansion and herniation of the trophectoderm cells from the zona at which time they were placed individually in 20 μl of G-MOPS medium (Vitrolife) under oil for biopsy. Applying gentle suction with the biopsy pipette (Cook Australia, Eight Mile Plains, Qld, Australia), trophectoderm cells were encouraged to herniate from the zona. Four to five trophectoderm cells were dissected from each of the blastocysts using four laser pulses of 3 m duration. The different stages of blastocyst biopsy are presented in Figure 1.

The biopsied cells were placed immediately in RNAse–DNAse-free PCR tubes and treated as described for cleavage stage biopsy and directly sent to the Laboratory of Medical Genetics for diagnosis (Traeger-Synodinos *et al.*, 2003; Vrettou *et al.*, 2004). On the morning of day 6, the morphology and growth of the biopsied blastocysts were assessed. Unaffected blastocysts were transferred about 144 h after fertilization to the patient's uterus using a Wallace catheter (SIMS Portex Ltd). On day 10 after embryo transfer, patient's serum β-HCG was assayed to evaluate initiation of a pregnancy, and at 6 weeks of gestation, the pregnancy was confirmed by ultrasound diagnosis of fetal heart beat.

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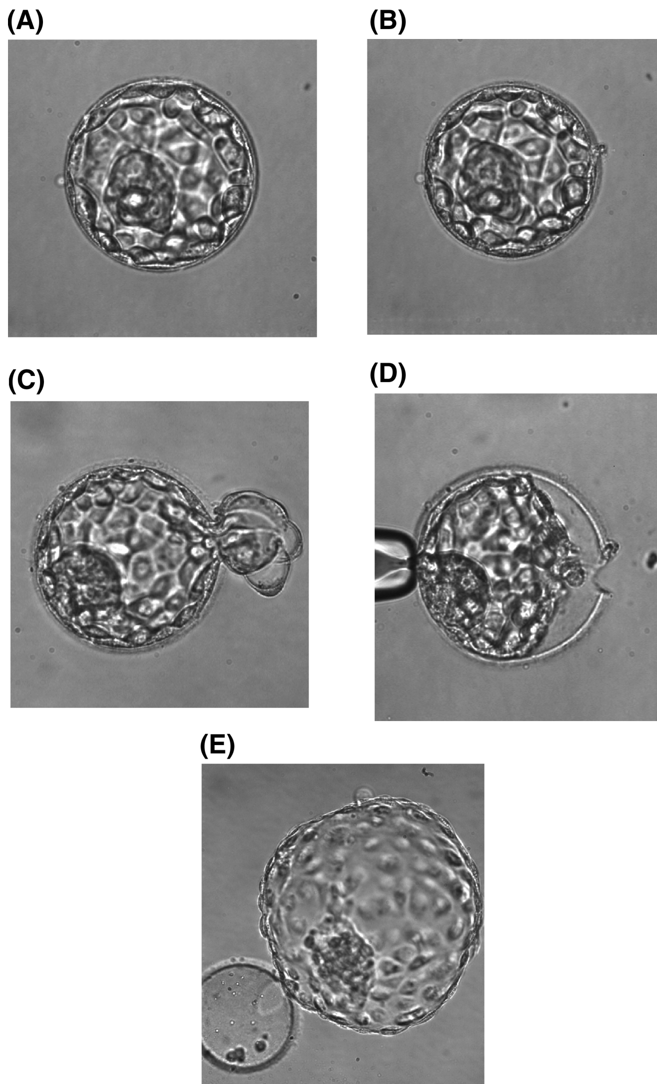

Figure 1. The process of blastocyst biopsy. (**A**) Expanding blastocyst on day 5. (**B**) Day 5 expanding blastocyst after zona opening using ZILOS-tk system. The site of zona opening is directly opposite the inner cell mass (ICM) and one trophectoderm cell has started herniating from the zona. (**C**) Following further incubation for 4 h, trophectoderm cells have herniated from the zona. These cells will be removed during the biopsy procedure. (**D**) Just after trophectoderm biopsy. The blastocyst collapses. The ICM may be seen intact and distant from the site of the biopsy procedure. (**E**) Following further incubation for 24 h after biopsy, the blastocyst has grown, expanded and hatched from the zona.

Genetic analysis

Cell samples derived from either cleavage stage or blastocyst stage biopsy were treated in exactly the same manner. The PGD genotyping protocol involved a first-round multiplex PCR, simultaneously amplifying a region of the β-globin gene containing the disease-associated mutations, along with two unlinked microsatellite markers (GABRB3 and D13S314); the primers for the microsatellite sites were fluorescently labelled (Cy5.5). The first round PCR reaction was then subject to (i) analysis of β-globin genotypes using real-time PCR protocol and allele-specific hybridization probes using the LightCycler™ system 1.0 (Roche Diagnostics GmbH, Manheim, Germany) and (ii) microsatellite sizing on a Visible Genetics OpenGene™ System automatic DNA sequencer with Gene Objects software (Visible Genetics, High Wycombe, UK) to exclude chance contamination, as described previously (Vrettou *et al.*, 2004). Embryo genotypes with respect to the parental β-globin mutations were available within 5–6 h following receipt of the biopsy samples at the genetics laboratory. In all resulting pregnancies, chorionic villous prenatal diagnosis was performed at the Laboratory of Medical Genetics, Athens University, to confirm β-thalassaemia genotypes (Vrettou *et al.*, 2003) and to evaluate fetal karyotype.

Statistics

Statistical analysis of the results was performed with the use of Student's *t*-test (to compare the means of normally distributed data between groups), Fisher's exact test (to compare dichotomous variables) and Kruskal–Wallis test (to compare nondichotomous variables, e.g. number of transferred embryos within two groups); Fisher's exact was preferred (over chi-square test) due to the small number of subjects. Data are presented as mean ±. $P < 0.05$ was considered as significant.

Results

The mean ages of the women were comparable between the two study groups (Table I). Couples with infertility factor were seven in Group A and six in Group B. In Group A, 131 oocytes were fertilized and cultured with the aim of biopsy on day 3, and in Group B, 128 oocytes were fertilized and cultured with the aim of biopsy on day 5.

In Group A, 101 (77%) embryos reached the six- to eight-cell stage and were biopsied. Of the 101 blastomeres removed, 76 (75.2%) gave a PCR product representing both alleles allowing a reliable diagnosis. In the remaining 25 blastomeres, 15 gave a PCR product representing only one of the two alleles (ADO) and in 10 the PCR failed. The total number of embryos diagnosed as unaffected for β-thalassaemia syndromes was 47/76. Of the 47 unaffected embryos, 35 developed to the blastocyst stage and were available for transfer. In Group A, 19 of the 29 affected embryos also developed to the blastocyst stage and a further 12 blastocysts developed from 25 embryos in which diagnosis was incomplete (Figure 1). The total number of embryos in Group A that developed to the blastocyst stage was 66 (50.4%).

A total of 30 blastocysts were transferred (average number 3 ± 1.05) among the 10 cycles in Group A, 5 days after fertilization. Surplus unaffected blastocysts were frozen to provide the option of future transfers. Six patients had a positive serum β-HCG pregnancy 10 days after embryo transfer. All pregnancies were confirmed at 6 weeks by ultrasound diagnosis with eight fetal hearts (four singletons and two twins) representing a 26.7% implantation rate. In one twin pregnancy, one fetus was lost at 9 weeks and two ectopic singleton pregnancies discontinued. Four pregnancies were delivered at 37.75 ± 0.96 weeks of gestation and five babies were born.

In Group B, 128 fertilized eggs developed to a total of 60 blastocysts (46.9%), of which 53 blastocysts were biopsied on day 5 for the diagnosis of β-thalassaemia, and the additional seven blastocysts which developed by day 6, for practical reasons, were frozen for future use without biopsy. Of the 53 blastocysts that were biopsied in Group B, 50 (94.3%) gave complete genotype analysis with 26 blastocysts diagnosed as unaffected for β-thalassaemia syndromes. A successful

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Table I. Overall cycle data for Groups A and B for the diagnosis of β-thalassaemia syndromes

Indication	Group A	Group B	*P*-value
Total cycles	10	10	
Female age (years)	36.8 ± 2.82	35 ± 2.94	
Day of biopsy	D3	D5	
Day of embryo transfer	D5	D6	
Biopsy procedure			
Zona breaching	Laser	Laser	
Biopsy method	Blastomere	Trophectoderm	
Embryology			
Fertilized	131	128	
Biopsied	101	53	
Diagnosed	76	50	0.002
Unaffected	47	26	
Transferable at blastocyst	35	26	
Transferred	30	21	
Average number transferred	3 ± 1.05	2.1 ± 0.99	0.065
Frozen	5	5 (+7[a])	
Total blastocyst developed	66	60	0.329
Blastocysts affected	19	14	
Blastocyst not diagnosed	12	3	
Clinical outcome			
Cycles to embryo transfer	10	10	
HCG positive	6	6	
Ectopic	2		
Miscarriage		1	
Number of fetal sacs	6 (+ 2)	10	
Implantation rate (%)	26.7%[b]	47.6%[b]	0.107
Viable pregnancies	4	5	0.5
Premature delivery (25 weeks)	0	1	
Pregnancies to term	4	4	
Babies born	5	7	
Average week of full-term delivery	37.75 ± 0.95	35.25 ± 4.35	
Reduction to twins		2	
Spontaneous abortion of one fetus	1	1	

[a]Seven blastocysts (in two cycles) additionally developed on day 6 and were frozen unbiopsied for future use.
[b]The implantation rate has been calculated by the number of fetal sacs per embryo transferred.

diagnosis was available in a significantly larger population of embryos in Group B (50/53) compared with Group A (76/101) ($P = 0.002$).

On the morning of day 6, the morphology and growth of biopsied blastocysts were assessed. All blastocysts had re-expanded and had started to herniate through the biopsy hole or had totally hatched from the zona (Figure 1).

A total of 21 blastocysts (average number 2.1 ± 0.99) were transferred among the 10 cycles in Group B, 6 days after fertilization. Surplus unaffected blastocysts were frozen to provide an option of future transfer. Six patients had a positive serum β-HCG pregnancy 10 days after embryo transfer. All pregnancies were confirmed at 6 weeks of gestation by ultrasound diagnosis with 10 fetal hearts (four singletons and two triplets), representing a 47.6% implantation rate, which according to the Fisher's exact test was not significantly different to the implantation rate for Group A (26.7%). One singleton pregnancy spontaneously aborted and one triplet pregnancy was reduced to twins. One singleton pregnancy was prematurely delivered at 25 weeks and four pregnancies were delivered with caesarean section at 35.25 ± 4.35 weeks, with seven babies born.

Prenatal diagnosis of pregnancies from Groups A and B confirmed an unaffected status with respect to β-thalassaemia and demonstrated normal karyotypes in all fetuses.

Discussion

The overall success of PGD, a healthy unaffected baby, requires both accurate genetic diagnosis and a positive outcome to all aspects of assisted reproduction technologies. The present limitations with respect to genetic diagnosis for monogenic diseases based on single blastomere analysis using PCR-based protocols led us to investigate whether the success of the genetic diagnosis could be improved by using trophectoderm biopsies of blastocysts without compromising the potential of pregnancy outcome in PGD cycles. Thus, this pilot study was designed to compare the outcome of PGD cycles for the diagnosis of β-thalassaemia based on either cleavage stage biopsy and day 5 blastocyst transfer or trophectoderm biopsy and day 6 blastocyst transfer.

The genetic diagnosis of monogenic disorders using PCR-based techniques to analyse single blastomeres faces several inherent problems, including complete failure of amplification or failure of one allele to amplify (ADO) (Kanavakis and Traeger-Synodinos, 2002; Sermon *et al.*, 2004). Over the last few years, there have been major advances in the PCR-based analytical methods applied to single cells, and the accuracy of nested PCR (Ray *et al.*, 1998) has been improved with the introduction of fluorescent PCR (Goossens *et al.*, 2000) and multiplex PCR (Dreesen *et al.*, 2000; Apessos *et al.*, 2001; Piyamongkol *et al.*, 2001; Vrettou *et al.*, 2004).

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These protocols minimize PCR failure and ADO and additionally monitor contamination.

Overall, the genotyping success in this and previous studies by the authors are in concordance with those of pooled data collected by the European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium for recessive monogenic diseases (Traeger-Synodinos *et al.*, 2003; Vrettou *et al.*, 2004; Harper *et al.*, 2006), whereby about 85–86% of biopsied single cells give a complete genotype. However, in this small sample of 10 cycles in Group A, although a PCR product was available in 90% of the biopsied embryos, only 75.2% of the embryos were definitively genotyped from single blastomere analysis due to ADO or preferential amplification in 15 samples (incidence of 15%). Among the 25 embryos that failed to give a result in Group A, 12 developed to blastocysts and would probably have been successfully genotyped if the approach of blastocyst biopsy had been applied.

Biopsy at the blastocyst stage provides the advantage that more cells can be removed for genetic analysis and, with respect to PGD for monogenic disorders, should potentially reduce the risk of ADO relative to analysis of just one cell from a cleavage-stage embryo. In this group of 10 cycles (Group B), the removal of four to five trophectoderm cells from each blastocyst achieved a complete genotype in 94.3% of the biopsied blastocysts. No ADO was apparent and the PCR failure that occurred in three samples may have been due to other technical difficulties such as nuclei loss from lysed cells due to manipulation prior to their placement in the PCR tubes. The preliminary data from the present study show that genetic analysis can be performed successfully on trophectoderm cells and because blastocyst stage biopsy can provide more cells for genetic analysis, the level of error in the diagnosis is minimized and thus the diagnosis is more reliable.

In the six- to eight-cell cleavage stage biopsy group, embryos were selected for biopsy based on morphological criteria (Rijnders and Jansen, 1998). However, embryo morphology on day 3 does not predict developmental potential nor does it evaluate the numerical chromosomal status (Magli *et al.*, 2000; Staessen *et al.*, 2004). Indeed, in our study, 22.9% of the cleavage stage embryos were unsuitable for biopsy on day 3 (<6 cells). Blastocyst biopsy provides a means for selecting embryos for biopsy that have at least demonstrated the potential of continued development under embryonic genomic control. Thus, selection is based on more objective criteria. In addition, embryos selected for biopsy on day 5 carry a lower risk of being aneuploid (Magli *et al.*, 2000; Staessen *et al.*, 2004), and although development to the blastocyst stage is not a guarantee of chromosomal normality, the majority of embryos that fail to continue in extended culture show multiple aneuploidies for chromosomes X, Y, 16, 18 and 21 (Magli *et al.*, 2000). In addition, observations suggest that the level of mosaicism in the trophectoderm is not higher than that seen in the inner cell mass of the blastocyst (Magli *et al.*, 2000; Evsikov and Verlinsky, 1998). The occurrence of mosaicism in blastocysts (either generalized mosaicism or a chromosomal dichotomy between inner cell mass and trophectoderm) could have consequences on the accuracy of PGD, but for monogenic disorders diagnosed with PCR-based methods, it is unlikely to lead to a misdiagnosis resulting in transfer of an affected embryo.

Regarding pregnancy outcomes after blastocyst transfer, the reported results are contradictory. A recent Cochrane review on the merits of blastocyst versus cleavage stage embryo transfer concluded that the current evidence fails to support a widespread change of practice from cleavage stage to blastocyst stage embryo transfer in couples undergoing IVF (Blake *et al.*, 2004), whereas a more recent well-designed prospective trial reported higher pregnancy and delivery rates after single blastocyst transfer compared with single cleavage stage embryo transfer (Papanikolaou *et al.*, 2006). Results of our laboratory, based on a prospective randomized trial, are in accordance with the Cochrane review conclusions (Pantos *et al.*, 2004b).

In the present study, embryos in both groups were incubated to the blastocyst stage before being transferred to the uterus. The reason for adopting this transfer policy for Group A subjects (instead of performing the transfer on day 4, following the day 3 biopsy) was that we wanted to observe the extended culture potential of day 3 biopsied embryos, as well as to compare implantation and pregnancy rates between transferred embryos of similar stage and thus avoiding bias due to different transfer day, although this does exclude the potential implantation of unaffected embryos had they been transferred on day 4.

The blastocyst development rates were comparable regardless of whether biopsy was performed at the early cleavage stage or at the blastocyst stage (Group A: 50.4%; Group B: 46.9%) and comparable with previous reports (Gardner and Lane, 1998; Gardner *et al.*, 1998a,b), with more than 90% of the blastocysts being formed by day 5 and the pregnancy rates (60% for both groups) not statistically different. In this small study, the implantation rates (Group A: 26.7%; Group B: 47.6%), although not statistically different, indicate that blastocyst biopsy may lead to a higher implantation rate. The above observation may be attributed to different transfer days [even though some investigators have reported increased implantation rates of embryos that are transferable on day 5 compared to day 6 (Barrenetxea *et al.*, 2005; Shapiro *et al.*, 2001) or simply to the small study sample size. Further studies are required to clarify this observation. The relatively high implantation rates observed in the present study are in accordance with 41% per biopsied fresh blastocyst reported by McArthur *et al.* (2005), although results from nonbiopsied day 6 blastocyst transfers previously reported by our laboratory were much lower: 12.4% in retrospective analysis of 4165 embryo transfers (Pantos *et al.*, 2004a) and 15.6% in a prospective analysis of 243 embryo transfers (Pantos *et al.*, 2004b). It has to be considered that the present cohort was small in size and that in selected cases (women 40 years old) the number of transferred embryos was maximized in accordance with national legislation (maximum of four embryos in patients aged $\geq$40). This practice resulted undoubtedly in increased rates of multiples, but it should be evaluated in view of the special requests of couples who were desperate for achieving a pregnancy following an expensive and stressful treatment.

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The promising results of the present pilot study allow us to consider with confidence the reduction in the number of transferred biopsied embryos.

The ability of blastocysts to implant after trophectoderm biopsy has been reported in animal studies (Gardner and Edwards, 1968; Gardner, 1971; Betteridge *et al.*, 1981; Monk *et al.*, 1988; Summers *et al.*, 1988), and to date, in humans, a few pregnancies have been reported following trophectoderm biopsy and PGD (Kokkali *et al.*, 2005; McArthur *et al.*, 2005; Tongyai *et al.*, 2004). The finding that pregnancy and implantation rates were not statistically different suggests that the implantation potential of the blastocyst following biopsy is not compromised. This is possibly because the trophectoderm cells removed are strictly extra-embryonic, potentially contributing only to placental tissues following implantation. It is predicted that removal of these cells avoids any risk of affecting the normal development of the fetus which is derived exclusively from the inner cell mass of the blastocyst. In addition, removal of four to five cells from a 100- or 150-cell blastocyst represents a lower proportion of loss than one cell from a six- to eight-cell embryo.

In summary, we have demonstrated that blastocyst biopsy is technically possible and all blastocysts survived the biopsy procedure as they continued to grow, develop and reconstitute their blastocoele cavity, with pregnancy rates comparable to a day 3 biopsy strategy. There is adequate time (up to 24 h) available for genetic testing prior to the time of transfer. In addition, PGD at the blastocyst stage is relatively more cost-effective and less labour intensive since only embryos competent to develop to the blastocyst stage are biopsied. Finally, the outcome of genotyping provides complete results in relatively more embryos.

Patients undergoing assisted conception and PGD procedures invest both financially and emotionally in the expected outcome of both a pregnancy and healthy offspring. With the availability of new embryology and molecular techniques, it is now possible for PGD laboratories to offer patients at genetic risk the transfer of developmentally competent embryos, unaffected by genetic disease. Trophectoderm biopsy at the blastocyst stage provides sufficient material for an effective and more reliable diagnosis in developmentally competent embryos. This preliminary study indicates that trophectoderm biopsy certainly does not compromise embryo implantation and pregnancy rates in PGD cycles, and further studies may even demonstrate an overall advantage of such an approach.

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