Article

Selective vitrification of euploid oocytes markedly improves survival, fertilization and pregnancy-generating potential

Dr Geoffrey Sher is a Fellow of the Royal College of Obstetrics and Gynaecology, a Fellow of the American College of Obstetrics and is qualified in Maternal and Fetal Medicine as a subspeciality. He is founder and Executive Medical Director of the Sher Institutes for Reproductive Medicine (SIRM) and is Clinical Professor of Obstetrics and Gynecology at the University Of Nevada School of Medicine. His research interests centre around factors that influence implantation following IVF, including: the role of ultrasound in the evaluation of oestrogen-induced endometrial proliferation, immunological factors and immunotherapy in IVF, and the role of HLA-G and the cytokine network in embryo–endometrial interaction.

Dr Geoffrey Sher

G Sher[1,2,6], L Keskintepe[1], T Mukaida[3], M Keskintepe[4], M Ginsburg[4], Y Agca[5], G Maassarani[1], A Bayrak[1]
[1]Sher Institutes for Reproductive Medicine; [2]Department of Obstetrics and Gynecology, University of Nevada School of Medicine, Reno, NV, USA; [3]Hart Institute, Hiroshima, Japan; [4]ReproCure LLC; [5]Pathobiology Department, University of Missouri, MO, USA
[6]Correspondence: Sher Institute for Reproductive Medicine, Las Vegas, 3121 S Maryland Parkway, Suite 300, Las Vegas, NV 89109, USA. Tel: +1 702 8929696; Fax: +1 702 8929666; e-mail: gsher@sherinstitute.com

Abstract

Enthusiasm for oocyte cryopreservation has been limited by poor pregnancy rates per thawed metaphase II (MII) oocytes (<4%) and low implantation rates per embryos. The reasons relate to technical limitations in the freezing process, and the fact that <40% of oocytes are euploid and unable to produce 'competent' embryos. Comparative genomic hybridization was performed on the first polar body (PB-1) of 323 MII oocytes retrieved from 16 donors. Of these, 111 were euploid, and were vitrified. Seventy-five of 78 vitrified oocytes (96%) survived warming and were fertilized using intracytoplasmic sperm injection. Thirty-one (41%) subsequently developed into expanded blastocysts, of which no more than two were subsequently transferred per uterus to 16 out of 19 prospective embryo recipients. Twelve of 19 (63%) recipients produced 17 healthy babies (eight singletons, three twins, and one set of triplets) One twin pregnancy miscarried in the late first trimester The birth rate per transfer of a maximum of two blastocysts to 16 recipients was 75%. The implantation rate per vitrified euploid oocyte was 27%. This study showed a six-fold improvement in pregnancy rate per cryopreserved oocyte over previous reports and a marked improvement in implantation rate. If independently validated, this approach could open the door to commercial egg cryobanking, significantly expanding women's reproductive choices.

Keywords: *CGH, implantation, oocyte, pregnancy, vitrification*

Introduction

In 1986, Chen reported the first pregnancy resulting from the transfer of embryos derived from previously cryopreserved oocytes (Chen, 1986). Since then, fewer than 350 births have been recorded in the scientific literature, although recent reports have been more encouraging (Isachenko *et al*., 2005; Kuwayama *et al*., 2005).

The success of human oocyte freezing is a function of the method of cryopreservation used and the 'quality' of the eggs selected. In the last few years, ultra-rapid embryo/oocyte vitrification has all but supplanted the conventional methods of cryopreservation. This has resulted in a significant improvement in post-thaw/warming oocyte survival and viability, as well as subsequent fertilization and pregnancy potential following embryo transfer (Kulleshova and Lopata, 2002; Kuwayama, 2007). Despite such enhancements, the highest reported pregnancy rate achieved per cryopreserved oocyte after fertilization and embryo transfer has been approximately 4% (Coticchio *et al*., 2007), thereby rendering the widespread introduction and/or commercialization of oocyte cryobanking both impractical and inadvisable.

It has recently been shown that approximately 60% of oocytes and embryos are aneuploid. These 'incompetent' oocytes are incapable of propagating healthy pregnancies, in addition to being far less likely to survive the cryopreservation process (Sher *et al.*, 2007b). In a previous study, full karyotyping of pre- and post-fertilized oocytes and embryos was performed using comparative genomic hybridization (CGH) performed on the first and second polar bodies (PB-1 and 2) biopsied from pre- and post-fertilized oocytes and blastomeres (day-3 embryos). The findings revealed that in young fertile women under age of 34, only 35% of mature metaphase II (MII) oocytes were euploid; >90% of euploid post-fertilized oocytes spawned euploid zygotes and embryos; 100% of aneuploid oocytes propagated aneuploid embryos; and embryos that failed to develop to the blastocyst stage were aneuploid in >95% of cases.

The present Western Institutional Review Board (WIRB)-approved study evaluated IVF outcome reported as implantation rates and live birth rates following the selective transfer of no more than two blastocysts derived through fertilization of previously vitrified euploid donor oocytes.

Materials and methods

Ovum donor recruitment

Sixteen oocyte donors under 35 years of age (mean ± SD = 29.4 ± 4.3) were recruited to this study between January 2006 and July 2007. After screening all oocyte donors for any significant medical, surgical and family history, they subsequently underwent comprehensive physical examinations and standard Food and Drug Administration-required laboratory testing that assessed for the presence of substance abuse, genetic diseases and sexually transmittable infections.

Ovarian stimulation, oocyte retrieval and polar body biopsy

Polar body biopsy and vitrification were completed in 3 h after oocyte retrieval. Following appropriate disclosure, each donor underwent ovarian stimulation as previously described (Fisch *et al.*, 2007). Human chorionic gonadotrophin (HCG), 10,000 IU (Profasi; Organon Pharmaceuticals, USA) was used to trigger ovulation in all cases. Oocyte retrieval was performed 34–37 h later using a transvaginal sonogram-guided needle. Conscious sedation was induced with intravenous Propofol. The surrounding cumulus oophorus was stripped from each oocyte and polar body biopsy was performed on all MII oocytes as previously described (Sher *et al.*, 2007a,b). MII oocytes were then individually vitrified using the cryoloop system (detailed below). Oocyte donor demographics and clinical criteria are presented in **Table 1**.

Comparative genomic hybridization (CGH)

Comparative genomic hybridization (CGH) for polar bodies was performed as described elsewhere (Sher *et al.*, 2007b).

Vitrification of MII oocytes

MII oocytes were kept at 37°C in modified human tubal fluid medium (mHTF; IVF Online, USA) supplemented with 10% synthetic serum supplement for 10–30 min until vitrification was conducted. MII oocytes were consecutively placed in different aliquots of vitrification media (VM), each comprising mHTF with a different concentration of dimethyl sulphoxide (DMSO) (2, 4, 6, 8, 10%) and ethylene glycol (2, 4, 6, 8, 10%) for 30, 30, 60, 60 and 90 s respectively. Finally, each MII oocyte was transferred to VM comprising mHTF + 20% DMSO + 20% ethylene glycol + 1 mol/l sucrose + 0.1 mol/l Ficoll for 30 s, and then placed on a 0.5 µm cryoloop. The latter was plunged directly into liquid nitrogen (–196°C) and placed in a 1-ml plastic vial for storage.

Oocyte warming

Individual cryoloops were removed from their plastic vials and plunged into media containing 1.5 mol/l sucrose at 37°C for 50 s, and then sequentially placed in sucrose, 1.0, 0.75, 0.5, 0.25, 0.125 and 0.0 mol/l respectively, for 0.5–2 min in each concentration at 37°C.

Oocyte fertilization and embryo transfer

Progesterone injections were initiated 6 days prior to intended embryo transfer, and vitrified oocytes were sequentially warmed one at a time until a total of three viable MII oocytes were available. Once oocytes were warmed and viability confirmed, they were kept in culture for 2 h prior to intracytoplasmic sperm injection (ICSI). All oocytes were cultured individually in 50 µl of Global One media (IVF online) and the drop covered with mineral oil. Cleaved embryos were individually cultured to the blastocyst stage in Global One medium at 37°C, in an environment of 6% CO_2, 5% O_2, 89% nitrogen and 95% humidity. Only those embryos that had developed to the expanded blastocyst stage by day 5 or 6 post-ICSI and exhibited well-developed inner cell masses and trophectoderms were deemed eligible for embryo transfer.

Table 1. Comparative genomic hybridization (CGH) results from first polar body biopsies performed on oocytes from 13 ovum donors.

Parameter	*Value*
Mean donor age in years ± SD	26.6 ± 4.4
No. of MII oocytes obtained	323
Mean no. of MII oocytes/donor ± SD	20.1 ± 8.3
CGH-normal oocyte *n* (%)	111 (34)
CGH-abnormal oocyte *n* (%)	191 (59)
CGH-equivocal oocytes[a] *n* (%)	21 (7)

[a]Ploidy undetermined.

Recruitment and preparation of embryo recipients

Nineteen embryo recipients were admitted to the study and all were treated free of charge. Only menopausal women and women with depleted or severely diminished ovarian reserve, as defined by a cycle day 3 FSH concentration of >15 mIU/ml in association with a plasma oestradiol concentration of <70 pg/ml and a past history of a poor ovarian response to ovarian stimulation (i.e. the production of ≤3 follicles following ovarian stimulation with >600 IU of FSH daily), were deemed eligible for participation in this study. Admittance was subject to physical and emotional criteria, which were determined through a detailed medical history, physical and psychological examinations, electrocardiogram and laboratory testing of both prospective parents. Full disclosure was made to all recipient couples, who were also counselled and then required to sign a written consent form. **Table 2** presents the demographic and clinical characteristics pertaining to the 19 embryo recipients whose ages ranged from 38 to 45 years (mean age 38.4 ± 3.6). All women had normal uterine cavities as assessed by preceding sonohysterography or hysteroscopy and had preceding ultrasound evidence of endometrial linings that measured ≥9 mm around the time of spontaneous or induced ovulation.

Hormonal treatment of recipients

An oestradiol valerate injection (4–8 mg) i.m. was administered every 3 days, starting with the onset of birth control pill-induced menstruation, until the plasma oestradiol concentration was stabilized. The goal of stabilizing the plasma oestradiol concentration at 500–1000 pg/ml in association with an endometrial thickness of ≥9 mm was achieved within 8–12 days in all cases. Subsequently, daily i.m. injections of 100 mg progesterone in oil were initiated. At the same time, pre-vitrified oocytes were warmed so as to obtain three viable oocytes available for ICSI using partner, or designated donor, spermatozoa. The fertilized oocytes were cultured for up to 6 days as previously described above.

Embryo transfer

Subject to patient choice and availability, no more than two blastocysts were transferred in each case. Supernumerary blastocysts were re-vitrified and cryobanked for future use at the sole discretion of the designated recipient and her partner. Embryo transfers were performed on day 6 of administration (day 5 of embryo development to the blastocyst stage). In the event of pregnancy, the prescribed oestrogen/progesterone regime was continued to week 10 of gestation. In all cases where pregnancy did not occur, or failed to survive, hormonal treatment was immediately stopped.

Statistical analysis

Differences between groups were evaluated with Student's *t*-test. Differences in rates and proportions among groups were evaluated by chi-squared tests and Fisher's exact test where appropriate. Significance was set at $P < 0.05$.

Table 2. Outcome of the transfer of blastocysts derived from the fertilization of warmed, previtrified euploid oocytes.

Parameter	*Value*
Prospective embryo recipients	19
Actual embryo recipients (i.e. no. of embryo transfers)	16
Mean recipient age in years ± SD	38.4 ± 3.6
Oocytes vitrified	111
Oocytes warmed	78
Oocytes that survived warming (%)	75 (96)
Cleaved embryos (%)	57 (76)
Blastocysts (%)	37 (65)
Blastocysts transferred	31
Blastocysts cryobanked (supernumerary)	6
Mean no. of blastocysts/embryo transfer ± SD	1.6 ± 0.4
Births/embryo transfer(%)	12/16 (75)
Births/woman (%)	12/19 (63)
Implantation rate/blastocyst (%)	19/31(61)
Implantation rate per warmed euploid oocyte (%)	19/72[a] (26)
Full-term pregnancies	12
Singletons	8
Twins	3
Triplets	1
Miscarriages	1

[a]Six supernumerary blastocysts of those derived from 78 vitrified/warmed oocytes were revitrified.

Results

Table 1 depicts the outcome of PB-1 CGH analysis of 323 mature (MII) previtrified/warmed oocytes (MII) obtained from 16 egg donors. A total of 111 (34%) were found to be euploid. Seventy-eight of these (70%) were subsequently warmed in preparation for ICSI, of which 75 (96%) survived the warming process, and 68 (91%) subsequently fertilized as shown by the development of two pronuclei within 16–18 h following ICSI. Fifty-seven (85%) of 67 viable warmed oocytes developed into cleaved embryos, of which 37 (65%) developed to the expanded and well differentiated blastocyst stage. Sixteen of 19 (84%) potential recipients underwent embryo transfer with no more than two blastocysts (mean = 1.2 ± 0.6). In three cases (16%), due to incompatibility in oocyte/recipient match, no viable blastocysts were generated for embryo transfer and in six cases at least one supernumerary embryo was re-vitrified and banked for future use by the designated couple. Twelve of the 19 (63%) potential recipients and 12 of the 16 women (75%) who underwent embryo transfer achieved live births. There were eight singleton pregnancies (67%), three (25%) sets of twins, and one (8%) triplet pregnancy. The implantation rate per blastocyst transfer was 61% (19/31). The transfer of 31/37 blastocysts derived from 78 warmed oocytes (six supernumerary blastocysts were cryobanked) resulted in 17 babies born and two concepti (twins) lost through one first trimester miscarriage, for an implantation rate per warmed, euploid oocyte of 27% (19/71).

Table 3 compares vitrified oocytes identified as euploid and aneuploid, and fresh donor oocytes that were collected during the study period. Euploid oocytes exhibited a higher survival rate (96%) than aneuploid oocytes (83%). Fertilization (by ICSI) rates were 93% (67/72) for vitrified euploid oocytes, 76% (47/62) for vitrified aneuploid oocytes and 87% (109/126) for fresh donor oocytes. In the euploid vitrified group, 1% of oocytes underwent abnormal fertilization 18 h after ICSI, whereas for aneuploid oocytes and fresh donor oocytes abnormal fertilization rates were 8 and 4% respectively. The cleavage rates on days 2 and 3 were better in vitrified euploid oocytes (85%) and fresh donor oocytes (92%) than in vitrified aneuploid oocytes (28%). Blastocyst development rates were similar (65 and 56%) for vitrified euploid oocytes and fresh donor oocytes, but superior to vitrified aneuploid oocytes (39%).

Discussion

Hitherto, results using cryopreserved oocytes have been rather disappointing (Boldt *et al.*, 2006). Reported oocyte survival rates per cryopreserved MII oocyte range from 60 to 85%, fertilization rates from 62 to 87%, and implantation rates per embryo from 13.6 to 27% (Boldt *et al.*, 2006; Coticchio *et al.*, 2007; De Santis *et al.*, 2007; Gook and Edgar, 2007). Even more alarming is the fact that the reported potential of a cryopreserved MII oocyte to survive thawing/warming with the ability to fertilize and, following embryo transfer, to be able to propagate a viable pregnancy is only about 4% (Coticchio *et al.*, 2007). Such statistics have heretofore made the recommendation of oocyte cryobanking to women seeking fertility preservation both injudicious and disingenuous.

The results reported in this study represent a six-fold improvement in the implantation rate per cryopreserved euploid MII oocyte. If corroborated through independent studies, these findings could open the door to widespread egg banking for fertility preservation and in the process, significantly expand the reproductive choices available to women. This will also be of obvious benefit to the thousands of women requiring treatments such as ovarian surgery, radiation, or chemotherapy, which often irreparably compromise oocyte viability. Reliable oocyte banking would also present the opportunity to temporarily store oocytes as a back-up option in case certain unforeseen problems arise during an IVF cycle, such as an absence or paucity of viable spermatozoa. In addition, the cryopreservation of pre-fertilized oocytes avoids the ethical concerns some patients may have with regard to the cryopreservation of embryos

It is important to mention that mature human oocytes have great heterogeneity in the distribution and organization of cytoplasmic organelles and demonstrate considerable variability in membrane water permeability. Both of these characteristics can profoundly influence their viability and 'competence', as

Table 3. Vitrified/warmed oocyte distribution, survival, fertilization and developmental competence by ploidy.

Parameter	*Euploid*	*Aneuploid*	*FDO*[1]	P-*value*
Oocyte donors	13	13	13	–
Mean donor age in years ± SD	26.6 ± 4.4	26.6 ± 4.4	25.8 ± 5.2	–
Metaphase II oocytes warmed	75	75	–	–
Survival (%)	72 (96)[a]	62 (83)[b]	–	0.05
Normal fertilization (%)	67 (93)	47 (76)	109/126 (87)	–
Abnormal fertilization (%)	1 (1)[c]	5 (8)[d]	5 (4)[c]	0.05
Degenerated oocytes (%)	1 (1)	2 (3)	2 (2)	–
Cleaved embryos (%)	57 (85)[e]	13 (28)[f]	100 (92)[g]	0.03
Blastocysts (%)	37 (65)[h]	5 (39)[i]	56 (56)[h]	0.05

[a–i]In each row, different superscript letters denote statistically significant differences; [1]FDO = fresh donor oocytes that were collected and injected during the same time period; no comparative genomic hybridization was performed on these.

well as the success of the oocyte cryopreservation process. When compared with other cell varieties, the oocyte, because of its spherical shape, has the lowest surface area-to-volume ratio (Wright *et al.*, 2004). Oocytes also have reduced permeability to cryoprotective additives (CPA) as compared with zygotes and embryos (Jackowski *et al.*, 1980). Zech *et al.* (2005) demonstrated that the creation of an opening in the zona pellucida of human blastocysts prior to vitrification improved cryopreservation. They suggested that vitrifying partially or completely hatched blastocysts enhances the embryo cryopreservation process. It may also be possible that biopsied embryos with openings in the zona pellucida could be more vulnerable to stress induced by conventional cryopreservation (Joris *et al.*, 1999; Magli *et al.*, 1999).

Oocyte cytoskeletal damage induced by increases in intracellular solute concentration along with intracellular ice formation represent the main reasons for cell damage during cryopreservation, and are more likely to occur with 'conventional' freezing (Borini *et al.*, 2007). Ice formation (the more detrimental factor) can in large part be avoided by reducing intracellular water content by inducing cell dehydration. The recent introduction of ultra-rapid vitrification of both oocytes and embryos has yielded much improved results (Mukaida *et al.*, 2003, 2006; Takahashi *et al.*, 2005). With vitrification, the concentration of cryoprotectant is sufficient to prevent crystallization such that both ice and rising solute concentrations are avoided. However, one of the dangers of the vitrification process is that the very high concentration of cryoprotectant required can damage the oocyte (Coticchio *et al.*, 2007). It is for this reason that regulation of both the introduction and removal of cryoprotectant, as well as the maintenance of an optimal temperature, is critical to the efficacy and safety of the process. The required concentration can be achieved through very rapid cooling and even more rapid warming (Gook and Edgar, 2007). The creation of an artificial slit at the zona pellucida during oocyte and/or embryo biopsy might in fact accelerate and improve subzonal dispersion of cryoprotectants at 37°C, as demonstrated for human blastocyst (Hiraoka *et al.*, 2007; Ge *et al.*, 2008). Recent reports (Kuwayama *et al.*, 2005; Antinori *et al.*, 2007; Cobo *et al.*, 2008) have confirmed prior reports that vitrified oocytes have an improved survival rate as compared with conventionally frozen oocytes, and that overall pregnancy rates are likewise higher. The implantation rates reported in the literature with unselected vitrified oocytes range from 11 to 13% (Kuwayama *et al.*, 2005; Antinori *et al.*, 2007), whereas the present study reports 26% implantation rate with CGH-selected vitrified/warmed oocytes.

A previous study has reported on the ability to fully karyotype all 23 chromosomes in the mature MII oocyte through the performance of CGH on the PB-1. It also reported that it is possible with 95% confidence to recognize euploid blastocysts based on the oocyte of origin being euploid.

In the present study, the fertilization rate of vitrified/warmed euploid oocytes (93%) was comparable with fresh donor oocytes (87%), and superior to vitrified aneuploid oocytes (76%). However, the blastocyst development rate was better in vitrified euploid oocytes (65%) and fresh donor oocytes (56%) than vitrified aneuploid oocytes (39%).

This study reports a 61% implantation rate per transferred blastocyst derived from fertilized, warmed euploid oocytes. While this represents a major improvement over prior reported results, it is nevertheless much lower than the 80% rate previously reported for blastocysts derived from fresh (non-precryopreserved) euploid oocytes (Sher *et al.*, 2007a). It is concluded that while oocyte vitrification is much less traumatic than previous methods of cryopreservation, it still exacts a measurable toll on egg/embryo viability. The embryo implantation rates and live birth rates per oocyte in this study are considerably higher than any other method or study reported in the literature, so far as is known. The improved success rates reported in this study may be attributed to two factors: (i) identification through PB-1 CGH of euploid oocytes; and (ii) the use of ultra-rapid vitrification.

While this preliminary report makes a strong case for selectively vitrifying and storing euploid oocytes for fertility preservation, for the time being it should not supplant the vitrification of euploid embryos derived through conventional IVF.

References

Antinori M, Licata E, Dani G *et al.* 2007 Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. *Reproductive BioMedicine Online* **14**, 72–79.

Boldt J, Tidswell N, Sayers A *et al.* 2006 Human oocyte cryopreservation: 5-year experience with a sodium-depleted slow freezing method. *Reproductive BioMedicine Online* **13**, 96–100.

Borini A, Bianchi V, Bonu MA *et al.* 2007 Evidence-based clinical outcome of oocyte slow cooling. *Reproductive BioMedicine Online* **15**, 175–181.

Chen C 1986 Pregnancy after human oocyte cryopreservation. *Lancet* **1**, 884–886.

Cobo A, Kuwayama M, Pérez *et al.* 2008 Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertility and Sterility* **89**, 1657–1664.

Coticchio G, Bonu MA, Sciajno R *et al.* 2007 Truths and myths of oocyte sensitivity to controlled rate freezing. *Reproductive BioMedicine Online* **15**, 24–30.

De Santis L, Cino I, Coticchio G *et al.* 2007 Objective evaluation of the viability of cryopreserved oocytes. *Reproductive BioMedicine Online* **15**, 338–345.

Fisch JD, Keskintepe L, Ginsburg M *et al.* 2007 Graduated Embryo Score and soluble human leukocyte antigen-G expression improve assisted reproductive technology outcomes and suggest a basis for elective single-embryo transfer. *Fertility and Sterility* **87**, 757–763.

Ge HS, Zhou R, Zhang W, Lin JJ 2008 Impact of assisted hatching on fresh and frozen–thawed embryos: a prospective, randomized study. *Reproductive BioMedicine Online* **16**, 589–596.

Gook DA, Edgar DH 2007 Human oocyte cryopreservation. *Human Reproduction Update* **13**, 591–605.

Hiraoka K, Fuchiwaki M, Hiraoka K *et al.* 2007 Zona pellucida removal and vitrified blastocyst transfer outcome: a preliminary study *Reproductive BioMedicine Online* **15**, 68–75.

Isachenko V, Montag M, Isachenko E *et al.* 2005 Aseptic technology of vitrification of human pronuclear oocytes using open-pulled straws. *Human Reproduction* **20**, 492–496.

Jackowski S, Leibo SP, Maxur P 1980 Glycerol permeabilities of fertilized and infertilized mouse ova. *Journal of Experimental Zoology* **212**, 329–341.

Joris H, Van den Abbeel E, Vos AD, Van Steirteghem A 1999 Reduced survival after human embryo biopsy and subsequent cryopreservation. *Human Reproduction* **14**, 2833–2837.

Kuleshova LL, Lopata A 2002 Vitrification can be more favorable than slow cooling. *Fertility and Sterility* **78**, 449–454.

Kuwayama M 2007 Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method.

Theriogenology **1**, 73–80.
Kuwayama M, Vajta G, Ieda S, Kato O 2005 Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. *Reproductive BioMedicine Online* **11**, 608–614.
Magli MC, Gianaroli L, Fortini D *et al*. 1999 Impact of blastomere biopsy and cryopreservation techniques on human embryo viability. *Human Reproduction* **14**, 770–773.
Mukaida T, Oka C, Goto T, Takahashi K 2006 Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human blastocysts. *Human Reproduction* **21**, 3246–3252.
Mukaida T, Takahashi K, Kasai M 2003 Blastocyst cryopreservation: ultrarapid vitrification using cryoloop technique. *Reproductive BioMedicine Online* **6**, 221–225.
Sher G, Keskintepe L, Mukaida T *et al*. 2007a Selective vitrification of euploid oocytes markedly improves their post-warming viability and post-fertilization pregnancy generating potential, thereby opening the door to commercial egg banking. *Fertility and Sterility* **88**, S343.
Sher G, Keskintepe L, Keskintepe M *et al*. 2007b Oocyte karyotyping by comparative genomic hybridization provides a highly reliable method for selecting 'competent' embryos, markedly improving in vitro fertilization outcome: a multiphase study. *Fertility and Sterility* **87**, 1033–1040.
Takahashi K, Mukaida T, Goto T, Oka C 2005 Perinatal outcome of blastocyst transfer with vitrification using cryoloop: a 4-year follow-up study. *Fertility and Sterility* **84**, 88–92.
Wright VC, Schieve LA, Reynolds MA *et al*. 2004 Assisted reproductive technology surveillance. *MMWR Surveillance Summaries* **30**, 1–20.
Zech NH, Lejeune B, Zech H, Vanderzwalmen P 2005 Vitrification of hatching and hatched human blastocysts: effect of an opening in the zona pellucida before vitrification. *Reproductive BioMedicine Online* **11**, 355–361.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 1 February 2008; refereed 21 February 2008; accepted 30 May 2008.