Reproductive BioMedicine Online (2012) **24**, 180–185

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Clinical validation of a closed vitrification system in an oocyte-donation programme

Dominic Stoop *, Neelke De Munck, Eleonora Jansen, Peter Platteau, Etienne Van den Abbeel, Greta Verheyen, Paul Devroey

Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium

* Corresponding author. *E-mail address:* dominic.stoop@uzbrussel.be (D Stoop).

Dominic Stoop took his medical degree in 2001 and completed his residency in obstetrics and gynaecology in 2007 at the Ghent University, Belgium. He joined the Centre for Reproductive Medicine of the Free University of Brussels in 2008 where he is currently a senior clinical fellow. He is an European Society of Human Reproduction and Embryology board-certified subspecialist in reproductive medicine and surgery. His main clinical and research interest is vitrification of human oocytes and embryos. He is clinically involved in oocyte donation through egg banking, fertility preservation and social egg freezing.

Abstract Controversy exists about the risk of microbiological contamination from direct contact with unsterile liquid nitrogen during oocyte vitrification. The aim of this observational study was to evaluate the effectiveness of oocyte vitrification using a high-security closed vitrification system in a donation programme. Oocyte vitrification was performed using CBS High Security closed straws (Cryo Bio System) with DMSO/ethylene glycol/sucrose as the cryoprotectant (Irvine Scientific freeze kit). A total of 123 vitrified metaphase-II oocytes were warmed in 20 recipient cycles (6.2 warmed oocytes per recipient); of these, 111 oocytes (90.2%) survived vitrification and warming. All surviving oocytes were microinjected and 86 (77.5%) were normally fertilized, of which 53 (61.6%) developed up to good-quality day 3. Ten embryo transfers resulted in a clinical pregnancy (50.0%) and an ongoing clinical pregnancy rate of 45%. Five revitrified embryos were warmed in three warming cycles (survival rate 100%). These transfers resulted in an additional ongoing twin pregnancy, leading to a cumulative ongoing pregnancy rate per patient of 50% (10/20). The ongoing implantation rate per warmed oocyte and per injected oocyte was 10.6% (13/123) and 11.7% (13/111). The present data demonstrate that oocyte vitrification using a closed vitrification device yields excellent oocyte survival, fertilization and embryo development.

© 2011, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: aseptic vitrification, closed carrier, cryopreservation, oocyte donation, vitrification

Introduction

Oocyte cryopreservation is only now becoming a routine procedure, even though the first successful pregnancies obtained after slow-rate freezing of eggs were pioneered more than two decades ago (Al-Hasani et al., 1987; Chen, 1986). Slow controlled-rate freezing allows oocytes to be cooled to very low temperatures while minimizing intracellular ice-crystal formation and the detrimental influences of

1472-6483/$ - see front matter © 2011, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.rbmo.2011.10.015

high solute concentrations. Kuleshova et al. (1999) reported the first birth from vitrified human oocytes after vitrification of 17 oocytes in open pulled straws. This technique represents ultra-rapid cooling, using high concentrations of cryoprotectants that solidify without the formation of ice. However, the perceived inefficiency and theoretical safety concerns limited its adoption to clinical situations in which no alternative was available, e.g. gonadotoxic therapy in single women.

In the last decade, the consensus grew that oocytes can be successfully cryopreserved by either slow-rate freezing or by vitrification through direct liquid-nitrogen contact (Loutradi et al., 2008; Oktay et al., 2006). Recently, several reports have demonstrated excellent clinical outcome from cryopreserved oocytes, especially through the open vitrification technique (Antinori et al., 2007; Cobo et al., 2008; Kuwayama et al., 2005; Nagy et al., 2009). Moreover, prospective randomized trials concluded that the outcome of vitrified–warmed oocytes was not inferior to fresh oocytes with regard to fertilization and embryo development and that comparable ongoing pregnancy rates were achieved (Cobo et al., 2010a; Rienzi et al., 2010). It is noteworthy that all studies concluding the non-inferiority of vitrified oocytes as compared with fresh oocytes have been performed with open devices.

A controversial issue remaining is the possibility of cross-contamination through direct contact of the vitrification sample with non-sterile liquid nitrogen in open devices (Bielanski et al., 2000, 2003; Bielanski and Vajta, 2009; Fountain et al., 1997; Tedder et al., 1995). Therefore, methods have been developed to prevent potential contamination. Sterilization of small amounts of liquid nitrogen during vitrification has been described as a feasible technique, while storage can be performed in the vapour phase of nitrogen (Cobo et al., 2010b; Eum et al., 2009; Parmegiani et al., 2010). Alternatively, open carriers can be placed in a hermetically sealed container before being plunged into the liquid nitrogen. This double-bagging technique (straw-in-straw method) appears to result in good post-warming embryo development in pronuclear oocyte and embryo vitrification (Kuleshova and Shaw, 2000; Vajta et al., 1998).

More recently, closed carriers have been introduced to deal with the above-mentioned concerns, allowing both vitrification and storage in the same device. Data obtained with closed vitrification systems such as CryoTip are scarce (Smith et al., 2010). Many authors share the opinion that the decreased cooling rate observed in closed devices due to isolation of the vitrifying samples potentially reduces the efficiency of oocyte vitrification. These assumptions are supported by data that suggest that the oocyte ultrastructure is better preserved with an open carrier (Bonetti et al., 2011). A recent randomized trial (Paffoni et al., 2011) comparing an open (CryoTop) versus a closed (CryoTip) device resulted in lower fertilization and cleavage rates in the latter. However, no prospective randomized trial has been performed comparing clinical outcomes. Moreover, data on clinical outcome after closed oocyte vitrification are limited. The main purpose of this study is to evaluate the clinical efficiency of vitrification using an aseptic closed device (CBS High Security straws; Cryo Bio System). A prospective observational study was performed in an oocyte-donation programme.

Materials and methods

This prospective, observational study had the approval of the ethical committee of the University hospital of the Vrije Universiteit Brussel (U. Ref. 2009/226). All donors were healthy women under the age of 35 and were screened and tested according to the Belgian regulations. Twenty recipients were included in the study. All participants fulfilled the admittance criteria and couples signed an informed consent.

Multifollicular ovarian stimulation

For all donors, a gonadotrophin-releasing hormone (GnRH)-antagonist protocol with recombinant FSH was used as previously described (Papanikolaou et al., 2005a,b). On day 2 of the menstrual cycle (day 1 of the stimulation) daily injections of recombinant FSH, follitropin β (Puregon; MSD, Oss, The Netherlands) was initiated. On day 7 of the cycle (day 6 of the stimulation) s.c. administration of GnRH antagonist ganirelix (Orgalutran; MSD) was started at a daily dose of 0.25 mg. From day 7 of the cycle onwards, ovarian ultrasound scans to assess follicular growth and blood sampling for oestradiol, progesterone, FSH and LH concentrations were performed to monitor and control follicular growth. Oocyte retrieval by transvaginal needle aspiration was performed 36 h after ovulation triggering with triptoreline 0.2 mg injection (Decapeptyl; Ipsen, Belgium).

Preparation of recipients

Preparation of recipients was performed using a standard protocol of GnRH agonist, oestrogen and progesterone. Busereline (Suprefact; Sanofi-Aventis, Belgium) was started in the midluteal phase of the cycle preceding the embryo-transfer cycle, at a daily dosage of 0.6 mg. After confirming down-regulation, by measuring the serum oestradiol and progesterone concentrations, oestrogen was administered orally using oestradiol valerate (Progynova; Bayer) at 2 mg twice daily for 6 days, then increased to 2 mg three times a day for 7 days. Endometrial thickness was measured on day 13 of oestradiol valerate administration. If the endometrial thickness reached 7 mm, a daily administration of 600 mg progesterone (Utrogestan, Bessins, Belgium) was started the day after. Oocytes were warmed on day 2 of utrogestan administration.

Laboratory procedures

Vitrification was carried out 2 h after oocyte retrieval with the Irvine Scientific Vitrification Freeze Kit containing: (i) equilibration solution (ES): 7.5% (v/v) ethylene glycol + 7.5% (v/v) dimethylsulphoxide (DMSO) in an M-199 HEPES-buffered medium supplemented with 20% dextran serum supplement (DSS); and (ii) vitrification solution: 15% (v/v) ethylene glycol + 15% (v/v) DMSO + 0.5 mol/l sucrose. Denuded oocytes were placed in 25 μl human tubal fluid (HTF)-HEPES supplemented with 5% (v/v) human serum albumin (HAS; Vitrolife, Sweden) for 1 min at 37 °C. The HTF-HEPES droplet containing the oocyte was merged with an adjacent droplet of 25 μl ES and kept at room temperature for 2 min; this droplet was again merged with a second,

new adjacent droplet of 25 μl ES and again kept at room temperature for 2 min. Then they were placed in 50 μl ES for 10 min at room temperature. Finally they were incubated in two 50 μl vitrification solution droplets, 15 s each at room temperature, followed by loading the oocyte into a High Security straw with minimal volume (~1 μl) and thermosealing before plunging into liquid nitrogen.

For warming, the Irvine Scientific Vitrification Thaw Kit was used, with minor modifications. The kit comprised: (i) a thawing solution (TS) containing 1 mol/l sucrose in an M-199 HEPES-buffered medium supplemented with 20% DSS; (ii) a dilution solution (DS) containing 0.5 mol/l sucrose in an M-199 HEPES-buffered medium supplemented with 20% DSS; and (iii) a washing solution (WS) containing M-199 HEPES-buffered medium supplemented with 20% DSS. Each time the upper part of the High Security straw was cut off. The straw containing the oocyte was taken out of the liquid nitrogen and was immediately placed in 200 μl TS for 1 min at 37 °C, followed by 3 min in DS (50 μl) and two 5-minute washes in WS (50 μl), both at room temperature. A volume of 200 μl TS was used rather than 50 μl to ensure a sufficiently high warming rate and to prevent evaporation.

After washing, oocytes were put in individual 25 μl droplets of fertilization medium (Sage; Cooper Surgical) under oil and scored for morphological survival. Subsequently, they were cultured for 2 h in an incubator with 5% O_2, 6% CO_2 and 89% N_2 and morphological survival was checked again. After 2 h of incubation, intracytoplasmic sperm injection was performed on all surviving oocytes and they were put in cleavage medium (Sage; Cooper Surgical) until the day of transfer (day 3). If the transfer was performed during the week, surplus good-quality embryos were vitrified on day 3. If an elective day-3 transfer was performed during the weekend, surplus embryos were cultured until day 5 in blastocyst medium (Sage; Cooper Surgical) and good-quality blastocysts were vitrified on day 5 or day 6.

All warmed oocyte embryo-transfer cycles were performed on day 3. Embryo quality on day 3 was scored according to the following parameters: number and equality of blastomeres, rate of fragmentation, multinucleation of the blastomeres, and early compaction. Selection of embryos for transfer on day 3 was based on the above parameters, with preference for embryos, which showed the normal developmental pattern of early cleavage on day 1, 4 cells on day 2 and 8 cells on day 3, with minimal fragmentation and no multinucleation. A combination of these parameters resulted in a combined embryo quality score: EQ1 = excellent quality; EQ2 = good quality; EQ3 = fair quality; and EQ4 = poor quality. The EQ4 embryos were not transferred. A top-quality embryo (EQ1) was considered as having at least 8 cells on day 3, with ≤10% fragmentation, regular size of the blastomeres and absence of multinucleation. Surplus embryos were revitrified and transferred in warmed oocyte frozen embryo-transfer cycles. Vitrification was performed on either on day 3 or at blastocyst stage. Blastocyst quality was assessed according to the criteria of Gardner and Schoolcraft (1999).

Results

From January 2010 to December 2010, 20 couples consented to have an oocyte-donation treatment after aseptic oocyte vitrification (Table 1). The mean age of the recipients was 39.1 ± 6.2 years. The cryopreserved oocytes originated from 14 oocyte donors with a mean age of 26.4 ± 4.8 years. The mean number of oocytes allocated to a recipient was 6.2.

Laboratory outcome

A total of 123 metaphase II oocytes were warmed in 20 warmed oocyte embryo-transfer cycles, of which 111 (90.2%) survived after warming. Eighty-six survived oocytes were normally fertilized after intracytoplasmic sperm injection (77.5%). Table 2 summarizes embryo quality at cleavage stage on day 3. Of the 80 embryos, 36% were excellent quality and transferred and 22 (61.1%) of the embryos transferred were excellent quality. A total of 12 embryos were cryopreserved, of which four were at cleavage stage and eight were at blastocyst stage. Three patients had a warmed oocyte frozen embryo-transfer cycle: one double-embryo transfer at blastocyst stage and two transfers (one single and one double) at cleavage stage.

Clinical outcome

Warmed oocyte embryo-transfer cycles resulted in a clinical pregnancy rate and ongoing clinical pregnancy rate of 50% and 45% respectively (Table 3). One pregnancy ended in a miscarriage and two warmed oocyte embryo-transfer cycles resulted in a twin pregnancy. One of the three warmed oocyte frozen embryo-transfer cycles with transfer at the blastocyst stage resulted in an ongoing twin pregnancy. The implantation rate and ongoing implantation rate per warmed oocyte was 11.4 and 10.6% respectively (Table 4). Per injected oocyte the observed implantation rate and ongoing implantation rate was 12.6 and 11.7% respectively. Including warmed oocyte embryo-transfer and warmed oocyte frozen embryo-transfer cycles, an ongoing pregnancy was achieved in 50% of the recipients (9 fresh + 1 frozen/20 cycles).

Discussion

The present data demonstrate that oocyte vitrification using a closed system can be an effective procedure for vitrification. This study reports 90.2% oocyte survival, 77.5% fertilization rate per survived oocyte and 69.9% fertilization rate per warmed oocyte. This oocyte cryo-banking outcome is comparable to the data reported with open systems. Recently published observational data of egg-bank donation in open systems (Nagy et al., 2009) reported 86.5% (134/155) oocyte survival and 87.3% (117/134) fertilization rate of per survived oocyte, resulting in an overall 75.5% (117/155) fertilization rate per warmed oocyte.

Table 1 Donor and recipient characteristics.

Characteristic	*Donors (n = 14)*	*Recipients (n = 20)*
Age (years)	26.4 ± 4.8	39.1 ± 6.2
Oocytes per recipient	—	6.2

Values are mean ± SD or mean.

Table 2 Laboratory outcomes: warmed oocyte embryo transfer.

Parameter	*Outcome*
Warmed oocytes	123
Surviving oocytes	111 (90.2)
Fertilized oocytes	86 (77.5)
Embryos on day 3	80
Excellent quality	29 (36.3)
Good quality	24 (30.0)
Moderate quality	16 (20.0)
Poor quality	11 (13.8)
Embryos transferred	36
Recipients with embryo transfer	20 (100)
Embryos transferred	1.8 ± 0.4
Excellent quality	22 (61.1)
Good quality	11 (30.6)
Moderate quality	3 (8.3)

Values are *n*, *n* (%) or mean ± SD.

Table 3 Clinical outcomes.

Outcome	*Warmed oocyte embryo transfers*	*Warmed oocyte frozen embryo transfers*	*All transfers*
Warming cycles	20	3	23
Transfers	20	3	23
Clinical pregnancy rate	10/20 (50.0)	1/3 (33.3)	11/23 (47.8)
Ongoing pregnancy rate	9/20 (45.0)	1/3 (33.3)	10/23 (43.5)
Implantation rate	12/36 (33.3)	2/5 (40)	14/41 (34.1)

Values are *n* or *n*/total (%).

Table 4 Efficiency of aseptic oocyte vitrification.

Outcome	*Per warmed oocyte*	*Per injected oocyte*
Clinical pregnancy rate	11/123 (8.9)	11/111 (9.9)
Implantation rate	14/123 (11.4)	14/111 (12.6)
Ongoing pregnancy rate	10/123 (8.1)	10/111 (9.0)
Ongoing implantation rate	13/123 (10.6)	13/111 (11.7)

Values are *n*/total (%).

Only limited outcome data have been published regarding closed oocyte cryopreservation. Closed vitrification appeared to be a feasible technique as demonstrated in a prospective randomized trial with better survival, fertilization, embryo development and clinical pregnancy rates with closed vitrification (CryoTip) as compared with slow-rate freezing (Smith et al., 2010). In comparison with open vitrification, the closed system cannot compete in terms of cooling rate with an open sample that is surrounded by a thin film of cryoprotectant and directly exposed to liquid nitrogen (Vajta et al., 2009). However, since the warming rate is at least as important as the cooling rate in the process, the overall efficiency of closed vitrification is highly dependent on open warming (Seki and Mazur, 2008). Isachenko et al. (1998, 1999) demonstrated that the warming—cooling proportion is even more important than the absolute speed of cooling or warming. Therefore, as the warming rates are minimally affected by closed vitrification, closed vitrification is still likely to surpass the warming—cooling proportion of 1.3, considered to be the most effective for successful vitrification (Isachenko et al., 1998, 1999). Ultimately, a randomized trial comparing ongoing pregnancy rate per warmed oocyte after open and closed vitrification needs to be performed in order assess the impact of the thermo-insulating barrier.

In the present study, the type and concentration of cryoprotectants, exposure times and temperatures were identical to those described with open systems (Nagy et al., 2009; Rienzi et al., 2010). This demonstrates that successful vitrification can be achieved without the need to modify cryoprotectant exposure to counteract reduced cooling rates (Vanderzwalmen et al., 2009, 2010).

In view of the scarcity in the literature of outcome data with closed oocyte vitrification, the study centre performed a randomized trial comparing open and closed oocyte vitrification prior to the observational trial presented here. The study was designed as a superiority trial powered to detect a difference in oocyte survival. The sample size, based on the hypothesis of finding superior oocyte survival after open vitrification, was calculated as follows: with 47 oocytes randomized to each arm, there would be 80% power to detect a 25% difference (60 versus 85%) at the 5% level of significance. The study did not show a significant difference in oocyte survival with a mean survival rate of 91.2% (67/73) for the closed system versus 84.5% (60/71) for the open system (CryoTop) with donated sibling oocytes. The size of the study was limited as national guidelines advise the use of a closed system to ensure separate storage of tissues and cells.

Open vitrification can be performed aseptically by separating the cooling from storage. Filtration or UV exposure can ensure sterile conditions during cooling, while storage can be performed by placing the open straw in a pre-cooled straw sealed afterwards (Vajta et al., 2009). Storage of open devices in the vapour phase above liquid nitrogen remains controversial as this still carries a real risk of sample contamination (Grout and Morris, 2009). The data presented in the present study describe an easier method for vitrification with a device that has been successfully tested to guarantee aseptic conditions (Benifla et al., 2000; Letur-Könirsch et al., 2004).

Although the size of the data set is limited, the results are promising. Future randomized controlled trials need to further elucidate whether the present method of closed oocyte vitrification is equivalent to open vitrification

combined with aseptical precautional measures. The present data at least suggest that closed oocyte vitrification is a valuable and aseptic alternative for open oocyte vitrification.

Acknowledgements

The authors thank S Vitrier and her co-workers at the laboratory of the Centre for Reproductive Medicine for their dedicated work. The authors would also like to thank the research nurses P Ackermans, E Nulens, E Van Moer and D Baeck.

References

Al-Hasani, S., Dietrich, K., van der Ven, H., Reinecke, A., Hartje, M., Krebs, D., 1987. Cryopreservation of human oocytes. Hum. Reprod. 8, 696–700.

Antinori, M., Licata, E., Dani, G., Cerusico, F., Versaci, C., Antinori, S., 2007. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reprod. Biomed. Online 14, 72–79.

Benifla, J.-L., Letur-Könirsch, H., Collin, G., Devaux, A., Kuttenn, F., Madelenat, P., Brun-Vezinet, F., Feldmann, G., 2000. Safety of cryopreservation straws for human gametes or embryos: a preliminary study with human immunodeficiency virus-1. Hum. Reprod. 15, 2186–2189.

Bielanski, A., Vajta, G., 2009. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum. Reprod. 24, 2457–2467.

Bielanski, A., Nadin-Davis, S., Sapp, T., Lutze-Wallace, C., 2000. Viral contamination of embryos in liquid nitrogen. Cryobiology 40, 110–116.

Bielanski, A., Bergeron, H., Lau, P., Devenish, J., 2003. Microbial contamination of embryos and semen during long term banking in liquid nitrogen. Cryobiology 46, 146–152.

Bonetti, A., Cervi, M., Tomei, F., Marchini, M., Ortolani, F., Manno, M., 2011. Ultrastructural evaluation of human metaphase II oocytes after vitrification: closed versus open devices. Fertil. Steril. 95, 928–935.

Chen, C., 1986. Pregnancy after human oocyte cryopreservation. The Lancet 327, 884–886.

Cobo, A., Kuwayama, M., Perez, S., Ruiz, S., Ruiz, A., Pellicer, A., Remohi, J., 2008. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the cryotop method. Fertil. Steril. 89, 1644–1657.

Cobo, A., Meseguer, M., Remohi, J., Pellicer, A., 2010a. Use of cryo-banked oocytes in an ovum donation programme: a prospective randomized, controlled, clinical trial. Hum. Reprod. 9, 2239–2246.

Cobo, A., Romero, J., Pérez, S., de los Santos, M., Meseguer, M., Remohi, J., 2010b. Storage of human oocytes in the vapour phase of nitrogen. Fertil. Steril. 94, 1903–1907.

Eum, J., Park, J., Lee, W., Cha, K., Yoon, T., Lee, D., 2009. Long-term liquid nitrogen vapor storage of mouse embryos cryopreserved using vitrification or slow cooling. Fertil. Steril. 91, 1928–1932.

Fountain, D., Ralston, M., Higgins, N., Gorlin, J.B., Uhl, L., Wheeler, C., Antin, J.H., Churchill, W.H., Benjamin, R.J., 1997. Liquid nitrogen freezers: a potential source of microbial contamination of hematopoietic stem cell components. Transfusion 37, 587–591.

Gardner, D., Schoolcraft, W., 1999. In vitro culture of human blastocysts. In: Jansen, R., Mortimer, D. (Eds.), Towards Reproductive Certainty: Infertility and Genetics Beyond 1999. Parthenon Press, Carnforth, pp. 378–388.

Grout, B.W.W., Morris, G.J., 2009. Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer. Theriogenology 71, 1079–1082.

Isachenko, V., Perez-Sanchez, F., Isachenko, V., Grishchenko, V., Soler, C., 1998. Vitrification of GV-porcine oocytes with intact intracellular lipids: effect of the cryoprotectant saturation/dilution stepping, elevated temperature and cytoskeletal inhibitor. Cryobiology 36, 250–253.

Isachenko, V., Gorbunov, J.L., Isachenko, E., Ostashko, F., Bezugly, N., 1999. Some physical and technological aspects of GV porcine oocyte vitrification. Cryobiology 39, 35 (abstract).

Kuleshova, L., Shaw, J., 2000. A strategy for rapid cooling of mouse embryos within a double straw to eliminate the risk of contamination during storage in liquid nitrogen. Hum. Reprod. 15, 2604–2609.

Kuleshova, L., Gianaroli, L., Magli, C., Ferraretti, A., Trounson, A., 1999. Birth following vitrification of a small number of human oocytes. Hum. Reprod. 14, 3077–3079.

Kuwayama, M., Vajta, G., Kato, O., Leibo, S., 2005. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod. Biomed. Online 11, 300–308.

Letur-Könirsch, H., Collin, G., Devaux, A., Sifer, C., Kuttenn, F., Madelenat, P., Brun-Vezineet, F., Feldmann, G., Benifla, J.-L., 2004. Conservation of human embryos in staws: safety in terms of human immunodeficiency virus 1. Gynecol. Obstet. Fertil. 32, 302–307.

Loutradi, K., Kolibianakis, E., Venetis, C., Papanikolaou, E., Pados, G., Bontis, I., Tarlatzis, B., 2008. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil. Steril. 90, 186–193.

Nagy, Z.P., Chang, C.C., Shaprio, D.B., Bernal, D.P., Carlene, W.E., Mitchlell-Leef, D., Toledo, A.A., Kort, H.I., 2009. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertil. Steril. 92, 520–526.

Oktay, K., Pelin, A., Bang, H., 2006. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil. Steril. 86, 70–80.

Paffoni, A., Guarneri, C., Ferrari, S., Restelli, L., Nicolosi, A.E., Scarduelli, C., Ragni, G., 2011. Effects of two vitrification protocols on the developmental potential of human mature oocytes. Reprod. Biomed. Online 22, 292–298.

Papanikolaou, E., D'haeseleere, E., Verheyen, G., Van de Velde, H., Camus, M., Van Steirteghem, A., Devroey, P., Tournaye, H., 2005a. Live birth is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture: a randomized prospective study. Hum. Reprod. 20, 3198–3203.

Papanikolaou, E., Bourgain, C., Kolibianakis, E., Tournaye, H., Devroey, P., 2005b. Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes. Hum. Reprod. 20, 1541–1547.

Parmegiani, L., Accorsi, A., Cognigni, G., Bernardi, S., Troilo, E., Fillicori, M., 2010. Sterilization of liquid nitrogen with ultraviolet irradiation for safe vitrification of human oocytes and embryos. Fertil. Steril. 94, 1525–1528.

Rienzi, L., Romano, S., Albricci, L., Maggiulli, R., Capalbo, A., Baroni, E., Colamaria, S., Sapienza, F., Ubaldi, F., 2010. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum. Reprod. 25, 66–73.

Seki, S., Mazur, P., 2008. Effect of warming rate on the survival of vitrified mouse oocytes and the recrystalization of intracellular ice. Biol. Reprod. 79, 727–737.

Smith, G., Serafini, P., Fioravanti, J., Yadid, I., Coslovsky, M., Hassun, P., Alegretti, J., Motta, E., 2010. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil. Steril. 94, 2088–2095.

Tedder, R., Zuckermann, M., Goldstone, A., Hawkins, A., Fielding, A., Briggs, E., Irwin, D., Blair, S., Gorman, A., Patterson, K., Linch, D., Heptonstall, J., Brink, N., 1995. Hepatitis B transmission from contaminated cryopreservation tank. The Lancet 346, 137—140.

Vanderzwalmen, P., Ectors, F., Grobet, L., Prapas, Y., Panagiotidis, Y., Vanderzwalmen, S., Stecher, A., 2009. Aseptic vitrification of blastocysts from infertile patients, egg donors and after IVM. Reprod. Biomed. Online 19, 700—707.

Vanderzwalmen, P., Zech, N., Prapas, Y., Panagiotidis, Y., Papatheodorou, A., Lejeune, B., Jareño, D., Vanderzwalmen, S., Ectors, F., 2010. Closed carrier device: A reality to vitrify oocytes and embryos in aseptic conditions. Gynecol. Obstet. Fertil. 38, 541—546.

Vajta, G., Lewis, I.M., Kuwayama, M., Greve, T., Callesen, H., 1998. Sterile application of the Open Pulled Straw (OPS) vitrification method. Cryo-Letters 19, 389—392.

Vajta, G., Nagy, Z.P., Cobo, A., Conceicoa, J., Yovich, J., 2009. Vitrification in assisted reproduction: myths mistakes, disbeliefs and confusion. Reprod. Biomed. Online 19, 1—7.

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

Received 21 April 2011; refereed 7 September 2011; accepted 20 October 2011.