J Assist Reprod Genet (2016) 33:1247–1252
DOI 10.1007/s10815-016-0748-7

GAMETE BIOLOGY

Closed vitrification of human oocytes and blastocysts: outcomes from a series of clinical cases

Debra A. Gook[1,2] · **Boon Choo**[1] · **Harold Bourne**[1] · **Kelly Lewis**[1] · **David H. Edgar**[1,2]

Received: 22 September 2015 / Accepted: 18 January 2016 / Published online: 28 May 2016

Abstract
Purpose High survival rates and clinical outcomes similar to those from fresh oocytes and blastocysts have been observed with open oocyte vitrification systems. It has been suggested that the extremely fast cooling rates that are only achieved with open systems are necessary for human oocyte and blastocyst vitrification. However, there is a potential risk of introducing contamination with open systems. The aim of this study was to assess whether similar survival and subsequent implantation rates could be achieved using a closed vitrification system for human oocytes and blastocysts.
Methods Initially, donated immature oocytes that were matured in vitro were vitrified using the cryoprotectants ethylene glycol (EG) + dimethyl sulphoxide (DMSO) + sucrose and either a closed system (Rapid-i®) or an open system (Cryolock). The closed system was subsequently introduced clinically for mature oocyte cryopreservation cases and blastocyst vitrification.
Results Using in vitro matured oocytes, a similar survival was achieved with the open system of 92.4 % (73/79) and with the closed system of 89.7 % (35/39). For clinical oocyte closed vitrification, high survival rate of 90.5 % (374/413) and an implantation rate of 32.7 % (18/55) from the transfer of day 2 embryos was achieved, which is similar to fresh day 2 embryo transfers. Blastocysts have also been successfully cryopreserved using the Rapid-i closed vitrification system with 94 % of blastocysts having an estimated ≥75 % of cells intact and a similar implantation rate (31.5 %) to fresh single blastocyst transfers.
Conclusion Closed vitrification can achieve high survival and similar implantation rates to fresh for both oocytes and blastocysts.

Keywords Closed vitrification · Human · Oocyte · Blastocyst · Implantation rate · Births

Capsule Closed vitrification can achieve high survival and similar implantation rates to fresh for both human oocytes and blastocysts.

✉ Debra A. Gook
debra.gook@mivf.com.au

[1] Reproductive Services and Melbourne IVF, Royal Women's Hospital, Parkville, Victoria, Australia

[2] Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, Australia

Introduction

Vitrification has changed the face of ART in recent years for both oocytes and blastocysts with more emphasis on cryopreservation cycles than ever before even to the extent of contemplating freeze all cycles [1] and social oocyte cryopreservation. The previous variable results with slow freezing of blastocyst and oocytes [2] have now been superseded by more reproducible efficient vitrification procedures [3, 4]. Vitrification of donor oocytes has resulted in high survival (92 %) and implantation rates (39.9 %) similar to fresh oocytes [4, 5]. Similarly, high survival and implantation rates with blastocyst vitrification [3, 6] have convinced clinics to adopt single cryopreserved blastocyst transfers [7].

However, these high survival rates have been achieved by extremely rapid cooling in direct contact with liquid nitrogen (open), raising the issue of potential contamination during cooling and/or during storage [8, 9]. Although this risk may be theoretical in ART with no reports of contamination, there are concerns that the risk may increase for oocytes in long-term storage for fertility preservation, which is now common practice. Procedures have been developed to reduce the

potential risk [10–13] and a number of closed vitrification tools developed [14], but there has been a reluctance to use closed systems due to the impact of slower cooling rates on survival.

Although it might depend on the tool, all closed vitrification systems achieved slower cooling rates than open systems [14]. In addition, some carriers are also associated with slower warming rates which may be more critical for survival [15, 16]. To compensate for slower cooling, higher concentrations of cryoprotectants have been used [17, 18] but these formulations are not commercially available, limiting the application of the closed system. Direct comparisons of open and closed systems have demonstrated similar survival for blastocysts for both [19–23]. Similar studies with oocytes observed lower survival for the closed system [18, 24], suggesting improvements are necessary to adopt a closed system for oocytes.

Due to infection control measures in force at our hospital, no material could be stored in an open system under liquid nitrogen, prompting the evaluation of the closed Rapid-i vitrification tool.

This study examines initially whether high survival can be achieved for human oocytes vitrified using a closed system. The study subsequently reports the application of the procedure to a clinical oocyte cryopreservation program. We also report the outcomes of blastocyst vitrification using the same closed tool.

Materials and methods

Patients

Oocytes were cryopreserved from patients undergoing controlled ovarian stimulation cycles from June 2012 to June 2015. Although over 340 patients stored oocytes, only 54 have subsequently warmed oocytes. Patients requesting warming were infertility patients who had oocytes vitrified due to failure to retrieve sperm ($n=37$), objection to embryo cryopreservation ($n=8$), ovarian hyperstimulation ($n=3$), fertility preservation ($n=2$), social reasons ($n=2$), and egg donation ($n=2$).

Oocytes

Retrieved oocytes were denuded of cumulus and corona cells using hyaluronidase (20 IU/ml; Hylase, Sanofi Aventis Australia) in Quinn's Advantage HEPES-buffered medium (QHEPES; SAGE BioPharma, USA). Metaphase II oocytes were held in Quinn's Advantage Fertilisation medium (QFERT; SAGE BioPharma, USA) containing 4-mg human serum albumin (HSA)/ml (SAGE) for a minimum of 1 h between denuding and dehydration. This concentration of HSA was used throughout unless stated.

For evaluation and development of the technology, the only material available was oocytes deemed unsuitable for clinical procedures, i.e. germinal vesicle and metaphase I oocytes recovered following denuding for ICSI. Therefore, the initial evaluation of the procedure and comparison of open and closed systems was using immature oocytes unsuitable for ICSI which were cultured overnight in QFERT and only resulting MII oocytes were vitrified. Due to legal restrictions, no subsequent fertilisation or embryo development could be assessed with these oocytes.

Open versus closed oocyte vitrification

All cryoprotectants were purchased from Sigma, Australia. Vitrification and warming basal medium was QHEPES supplemented with 20 mg HSA/ml.

The in vitro matured metaphase II oocytes were exposed to equilibration solution; 7.5 % ethylene glycol (EG) + 7.5 % dimethyl sulphoxide (DMSO), at room temperature until the oocyte had re-expanded to approximately 80 % of initial volume. Re-expansion was achieved when no faceted sides were observed and the oocyte membrane was completely smooth. Due to known variability in membrane permeability which is exacerbated with culture, no standard time was used. Each oocyte was moved after re-expansion (range 4-12 min; $n=118$ oocytes) was complete. Using a small bore pipette to reduce dilution effects, the oocyte was moved to the vitrification solution; 15 % EG + 15 % DMSO + 0.5 M sucrose, at room temperature and within 1 min was loaded onto the vitrification tool and vitrified. For the Cryolock® (BioDiseno), the oocyte was loaded in minimal volume (<0.1 μL) on to the flat end and plunged directly into liquid nitrogen followed by covering with the cap (open). For the closed tool, the oocyte was loaded into the 30-nl hole of the Rapid-i™ (Vitrolife), the metal rod in the precooled sleeve removed, and the Rapid-i then dropped into a pre-cooled sleeve (in a liquid nitrogen bath). Care was taken to reduce liquid nitrogen vapour entering the sleeve prior to loading the Rapid-i by covering the opening. The sleeve was then sealed with an ultrasonic sealer. All oocytes were stored under liquid nitrogen for at least 7 days before warming.

Clinical application of closed oocyte vitrification

Metaphase II oocytes were vitrified using the closed tool (Rapid-i) only. From the assessment of re-expansion times in the initial study, a uniform time of 12 min in the ES solution was used for all clinical oocytes. Generally two oocytes were loaded onto each Rapid-i.

Oocyte warming

For the open tool, the cap was removed under liquid nitrogen and the Cryolock end plunged directly into first warming

solution (1.0 M sucrose) at 37 °C for 1 min. For the closed tool, the sleeve containing the Rapid-i was placed in a liquid nitrogen bath, the sealed end cut and the Rapid-i removed quickly and dipped directly into the first warming solution (same as above). The cryoprotectants were subsequently diluted by transferring the oocyte to 0.5 M sucrose at room temperature for 3 min and finally two washes in QHEPES at 37 °C for 3 min. For the open and closed comparison with the in vitro matured oocytes, initial survival was confirmed following overnight culture in QFERT. The vitrified clinical oocytes were warmed following the same procedure for the closed vitrified tool.

Clinical oocytes; fertilisation and embryo development

The following warming oocytes were incubated in QFERT for a minimum of 1 h before ICSI was performed. The ICSI procedure has been previously reported [25]. Oocytes were then cultured in QFERT and assessed for pronuclei at 16–18 h post injection and subsequently transferred to Quinn's Advantage Cleavage medium. Assessment of timing of syngamy and first cleavage has been previously reported [26], together with selection criteria for day 2 embryo transfer [27]. All remaining embryos suitable for cryopreservation were subsequently cryopreserved by slow cooling on day 2 using the 1.5 M PROH + 0.2 M sucrose embryo method previously reported [28]. For comparison, fresh single embryo transfers on day 2 during January 2013 and December 2014 were included. Implantation was defined as detection of an intrauterine fetal heartbeat.

Blastocyst development

Extended culture of fresh embryos was used where applicable in IVF/ICSI cycles within our ART program. Embryos were cultured in Quinn's blastocyst medium (QBlast) and excess good quality blastocysts on day 5 were vitrified. Medium was replenished for all other embryos and culture continued for reassessment on day 6. Blastocysts which subsequently developed on day 6 were also vitrified.

Blastocyst vitrification

It is prohibited in Australia to undertake experimentation on embryos used in clinical treatment. Therefore, the solutions tested by Vitrolife for use in conjunction with the tool were used for blastocyst vitrification. Excess blastocysts were vitrified on day 5 or 6 using the closed tool (Rapid-i) and the RapidVit™ Blast Kit solutions at 37 °C. The blastocyst was transferred to the G-MOPS holding solution and then in minimal volume moved to the Vitri 2 solution; 8 % EG + 8 % propanediol (PROH) for 2 min. The blastocyst was subsequently moved again in minimal volume to Vitri 3; 16 % EG + 16 % PROH + 0.65 M sucrose + ficoll and loaded at 30 s onto the Rapid-i.

Blastocyst warming

The solutions used were the RapidWarm™ Blast kit, all at 37 °C. The sleeve was opened as above and Rapid-i dipped immediately into 0.25 M sucrose; the blastocyst remained in this solution for 5 min and then moved to 0.125 M sucrose for 5 min followed by G-MOPS/albumin for 5 min. Blastocysts were cultured for a minimum of 2 h in QBlast before transfer. Cell counts to determine the proportion of cells surviving within the blastocyst could not be determined due to legal restrictions on embryos used in a clinical procedure and, therefore, the survival reported is an estimate and can be biased by the operator. Data presented is of single vitrified blastocyst transfers during January 2014 to March 2015. For comparison, fresh single day 5 blastocyst transfers during the same time period are included. Data was analysed by chi square.

Results

Open versus closed oocyte vitrification

There was no difference in survival between the open [92.4 % (73/79)] and closed [89.7 % (35/39)] vitrification of in vitro matured oocytes.

Clinical oocyte vitrification

The results for clinical oocytes are presented in Table 1 and are stratified according to female age at cryopreservation (<38 and ≥38). All metaphase II oocytes were vitrified irrespective of morphological appearance. Despite this, the post thaw survival rate was 90.5 % and was similar for both age groups. The fertilisation rate appeared relatively low (64.2 %) but two thirds of the patients used sperm recovered from repeated testicular biopsy for the ICSI procedures. A high proportion of the vitrified oocytes which fertilised subsequently cleaved (90.4 %) on day 2 and the fetal heart implantation rates for both age groups [<38 and ≥38 (34.2 %, 29.4 %) respectively] were not significantly different to our fresh implantation rates [32.3 % (426/1318) and 14.0 % (98/698)] for the same age groups. This corresponds to implantation rates per oocyte thawed of 4.1 % for <38 and 5.1 % for ≥38.

Blastocyst vitrification

A high proportion of blastocysts (94 %) survived with an estimated ≥75 % of cells intact irrespective of whether cryopreserved on day 5 (96.0 %) or day 6 (90.8 %) using the closed vitrification system (Table 2). The implantation rates for the

Table 1 Survival and clinical outcomes for closed vitrified oocytes

	<38	≥38	Total
Mean age at vitrification	32.2	40.6	34.7
Number of patients	39	15	54
Survival rate	90.5 % (285/315)	90.8 % (89/98)	90.5 % (374/413)
Fertilisation rate	66.3 % (189/285)	57.3 % (51/89)	64.2 % (240/374)
Proportion of cleavage on day 2	91.0 % (172/189)	88.2 % (45/51)	90.4 % (217/240)
Number of transfers	31	13	44
Number of fresh embryos transferred	38	17	55
Implantation rate (number FH)	34.2 % (13)	29.4 % (5)	32.7 % (18)
Implantation rate per oocyte	4.1 % (13/315)	5.1 % (5/98)	4.3 % (18/413)
Clinical pregnancies	41.9 % (13/31)	38.5 % (5/13)	40.9 % (18/44)
Miscarriage	2 (6w, 10w)	2 (8w, 10w)	4
Fresh			
Implantation rate (number FH)	32.3 % (426/1318)	14.0 % (98/698)	

day 5 and 6 vitrified blastocyst were similar within both age groups (<38 and ≥38; Table 3) and not significantly different to the fresh single blastocyst implantation rates for the same age groups [36.3 % (353/972) and 21.5 % (80/372)].

Discussion

The results presented have clearly established that closed vitrification with the Rapid-i tool can achieve high survival and implantation rates for both oocytes and blastocysts. Oocyte survival using the closed Rapid-i tool was similar to reports for open systems using the same cryoprotectants [4, 29–34]. Limited previous experience with a variety of closed systems (not Rapid-i) has generated survival rates ranging from 60 to 90 % [18, 24, 35, 36]. The super cooled air in the Rapid-i sleeve cools at 1220 °C/min [22] which is more than 50-fold slower than the rate when using direct contact with liquid nitrogen 69,000 °C/min [37] but survival was similar for both direct contact and the Rapid-i. The comparison of the open and closed tools using the same dehydration process in the present study indicates that, at least with the Rapid-i tool, there is no requirement to increase cryoprotectant concentrations during the dehydration process to compensate for the slower cooling rate, as suggested by previous studies [18]. It can be assumed from the identical process for the warming that the warming rate is the same for both the Cryolock and Rapid-i (117,500 °C/min) which Seki and Mazur (2012) suggest to be the more critical factor for survival. Critical for achieving high survival with the Rapid-i is eliminating vapour exposure during handling of the Rapid-i and accumulation inside the straw, which are likely to result in a slower cooling rate and may also initiate ice nucleation.

Although the fertilisation rate in the present study is lower than previously reported for our fresh oocytes and slow frozen oocytes [38], this is probably due to the change from use of donor sperm in cases of failed testicular retrieval [38] to fertilisation with testicular sperm obtained from repeated attempts at testicular biopsy in the majority of cases in the present study. The fertilisation rate for fresh oocytes using testicular sperm in our clinic during the same time period is 61.4 % (1227/1998). This is not significantly different to fertilisation rate for the vitrified oocytes, which supports the concept that this is a likely confounding factor in the fertilisation rate with the vitrified oocytes in present study. The high proportion of oocytes vitrified in the closed system which subsequently cleave and implant is similar to fresh oocytes indicating that the vitrification had no impact on subsequent development. This is reassuring but the numbers are small and perinatal outcomes need to be compared. No birth abnormalities were reported.

High survival with the Rapid-i was also observed for blastocysts regardless of the day of vitrification which was similar

Table 2 Blastocyst survival following closed vitrification

Estimated proportion of cells intact	Survival rate for day 5	Survival rate for day 6	Total
<50 %	2.3 % (16/698)	5.5 % (25/456)	3.5 % (41/1154)
50-74 %	1.7 % (12/698)	3.7 % (17/456)	2.5 % (29/1154)
≥75 %	96.0 % (670/698)	90.8 % (414/456)	93.9 % (1084/1154)

Table 3 Implantation rate of single blastocyst transfers following closed vitrification

	<38	≥38	Total
Vitrified day 5	35.9 % (160/446)	25.4 % (34/134)	33.5 % (194/580)
Vitrified day 6	31.4 % (89/283)	21.9 % (25/114)	28.7 % (114/397)
Total	34.2 % (249/729)	23.8 % (59/248)	31.5 % (308/977)
Fresh	36.3 % (353/972)	21.5 % (80/372)	32.2 % (433/1344)

to results obtained for open vitrification on day 5 and day 6 [3]. Comparisons of open and closed vitrification of blastocysts have all achieved similar survival and implantation rates [19–23] for both. In these comparisons, two closed tools have been used; the CBS straw [19, 21] and the Rapid-i [20, 22, 23]. High survival of blastocysts with closed vitrification in 15 % EG, 15 % DMSO and 0.5 M sucrose, at least with the Rapid-i [20, 23], indicates, as for oocytes, that there is no requirement for increased concentrations of cryoprotectants. The EG/DMSO combination together with a slightly higher sucrose concentration (0.65 M) and ficoll with the Rapid-i also achieves high survival and implantation rates for blastocysts [22]. Similarly, the combination of 16 % EG, 16 % PROH, 0.65 M sucrose and ficoll, used in the present study, has also achieved high rates indicating that adherence to the EG/DMSO combination is not mandatory with the Rapid-i. The high survival in the present study also supports the concept that artificial collapse of the blastocyst is not necessary.

The results presented in the present study confirm that closed vitrification is an efficient reproducible approach for both oocyte and blastocyst vitrification, which minimises the possible risk of contamination.

References

1. Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. Hum Reprod. 2014;29(12):2794–801.
2. Edgar DH, Gook DA. A critical appraisal of cryopreservation (slow cooling versus vitrification) of human oocytes and embryos. Hum Reprod Update. 2012;18:536–54.
3. Cobo A, de los Santos MJ, Castello D, Gamiz P, Campos P, Remohi J. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil Steril. 2012;98(5):1138–46. e1.
4. Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod. 2010;25(9):2239–46.
5. Cobo A, Remohi J, Chang CC, Nagy ZP. Oocyte cryopreservation for donor egg banking. Reprod Biomed Online. 2011;23(3):341–6.
6. Liebermann J. Vitrification of human blastocysts: an update. Reprod Biomed Online. 2009;19 Suppl 4:4328.
7. Devine K, Connell MT, Richter KS, Ramirez CI, Levens ED, DeCherney AH, et al. Single vitrified blastocyst transfer maximizes liveborn children per embryo while minimizing preterm birth. Fertil Steril. 2015;103(6):1454–60. e1.
8. Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C. Viral contamination of embryos cryopreserved in liquid nitrogen. Cryobiology. 2000;40:110–6.
9. Tedder RS, Zuckerman MA, Goldstone AH, Hawkins AE, Fielding A, Briggs EM, et al. Hepatitis B transmission from contaminated cryopreservation tank. Lancet. 1995;346(8968):137–40.
10. Bielanski A, Vajta G. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum Reprod. 2009;24:2457–67.
11. Cobo A, Romero JL, Perez S, de Los Santos MJ, Meseguer M, Remohi J. Storage of human oocytes in the vapor phase of nitrogen. Fertil Steril. 2010;94(5):1903–7.
12. Parmegiani L, Accorsi A, Cognigni GE, Bernardi S, Troilo E, Filicori M. Sterilization of liquid nitrogen with ultraviolet irradiation for safe vitrification of human oocytes or embryos. Fertil Steril. 2010;94(4):1525–8.
13. Parmegiani L, Accorsi A, Bernardi S, Arnone A, Cognigni GE, Filicori M. A reliable procedure for decontamination before thawing of human specimens cryostored in liquid nitrogen: three washes with sterile liquid nitrogen (SLN2). Fertil Steril. 2012;98(4):870–5.
14. Vajta G, Rienzi L, Ubaldi FM. Open versus closed systems for vitrification of human oocytes and embryos. Reprod Biomed Online. 2015;30(4):325–33.
15. Seki S, Mazur P. Effect of warming rate on the survival of vitrified mouse oocytes and on the recrystallization of intracellular ice. Biol Reprod. 2008;79(4):727–37.
16. Seki S, Mazur P. The dominance of warming rate over cooling rate in the survival of mouse oocytes subjected to a vitrification procedure. Cryobiology. 2009;59(1):75–82.
17. Vanderzwalmen P, Zech N, Prapas Y, Panagiotidis Y, Papatheodorou A, Lejeune B, et al. Closed carrier device: a reality to vitrify oocytes and embryos in aseptic conditions. Gynecol Obstet Fertil. 2010;38(9):541–6.
18. Papatheodorou A, Vanderzwalmen P, Panagiotidis Y, Prapas N, Zikopoulos K, Georgiou I, et al. Open versus closed oocyte vitrification system: a prospective randomized sibling-oocyte study. Reprod Biomed Online. 2013;26(6):595–602.
19. Chen Y, Zheng X, Yan J, Qiao J, Liu P. Neonatal outcomes after the transfer of vitrified blastocysts: closed versus open vitrification system. Reprod Biol Endocrinol. 2013;11:107.
20. Iwahata H, Hashimoto S, Inoue M, Inoue T, Ito K, Nakaoka Y, et al. Neonatal outcomes after the implantation of human embryos vitrified using a closed-system device. J Assist Reprod Genet. 2015;32(4):521–6.
21. Panagiotidis Y, Vanderzwalmen P, Prapas Y, Kasapi E, Goudakou M, Papatheodorou A, et al. Open versus closed vitrification of blastocysts from an oocyte-donation programme: a prospective randomized study. Reprod Biomed Online. 2013;26(5):470–6.
22. Desai NN, Goldberg JM, Austin C, Falcone T. The new Rapid-i carrier is an effective system for human embryo vitrification at both the blastocyst and cleavage stage. Reprod Biol Endocrinol. 2013;11:41.
23. Hashimoto S, Amo A, Hama S, Ohsumi K, Nakaoka Y, Morimoto Y. A closed system supports the developmental competence of human embryos after vitrification : closed vitrification of human embryos. J Assist Reprod Genet. 2013;30(3):371–6.
24. Paffoni A, Guarneri C, Ferrari S, Restelli L, Nicolosi AE, Scarduelli C, et al. Effects of two vitrification protocols on the developmental potential of human mature oocytes. Reprod Biomed Online. 2011;22:292–8.
25. Bourne H, Richings N, Harari O, Watkins W, Speirs AL, Johnston WIH, et al. The use of intracytoplasmic sperm injection for the treatment of severe and extreme male infertility. Reprod Fertil Dev. 1995;7:237–45.
26. Lawler C, Baker HW, Edgar DH. Relationships between timing of syngamy, female age and implantation potential in human in vitro-fertilised oocytes. Reprod Fertil Dev. 2007;19(3):482–7.
27. Edgar DH, Bourne H, Speirs AL, McBain JC. A quantitative analysis of the impact of cryopreservation on the implantation potential of human early cleavage stage embryos. Hum Reprod. 2000;15:175–9.
28. Edgar DH, Karani J, Gook DA. Increasing dehydration of human cleavage-stage embryos prior to slow cooling significantly increases cryosurvival. Reprod Biomed Online. 2009;19(4):521–5.

29. Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertil Steril. 2009;92(2):520–6.
30. Ubaldi F, Anniballo R, Romano S, Baroni E, Albricci L, Colamaria S, et al. Cumulative ongoing pregnancy rate achieved with oocyte vitrification and cleavage stage transfer without embryo selection in a standard infertility program. Hum Reprod. 2010;25(5):1199–205.
31. Garcia JI, Noriega-Portella L, Noriega-Hoces L. Efficacy of oocyte vitrification combined with blastocyst stage transfer in an egg donation program. Hum Reprod. 2011;26:782–90.
32. Trokoudes KM, Pavlides C, Zhang X. Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program. Fertil Steril. 2011;95(6):1996–2000.
33. Rienzi L, Cobo A, Paffoni A, Scarduelli C, Capalbo A, Vajta G, et al. Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. Hum Reprod. 2012;27:1606–12.
34. Sole M, Santalo J, Boada M, Clua E, Rodriguez I, Martinez F, et al. How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes. Hum Reprod. 2013;28(8):2087–92.
35. Stoop D, De Munck N, Jansen E, Platteau P, Van den Abbeel E, Verheyen G, et al. Clinical validation of a closed vitrification system in an oocyte-donation programme. Reprod Biomed Online. 2012;24:180–5.
36. De Munck N, Verheyen G, Van Landuyt L, Stoop D, Van de Velde H. Survival and post-warming in vitro competence of human oocytes after high security closed system vitrification. J Assist Reprod Genet. 2013;30(3):361–9.
37. Seki S, Mazur P. Ultra-rapid warming yields high survival of mouse oocytes cooled to −196 degrees C in dilutions of a standard vitrification solution. PLoS ONE. 2012;7(4):e36058.
38. Gook DA, Edgar DH. Implantation rates of embryos generated from slow cooled human oocytes from young women are comparable to those of fresh and frozen embryos from the same age group. J Assist Reprod Genet. 2011;28(12):1171–6.