J Assist Reprod Genet (2015) 32:521–526
DOI 10.1007/s10815-015-0431-4

ASSISTED REPRODUCTION TECHNOLOGIES

Neonatal outcomes after the implantation of human embryos vitrified using a closed-system device

Hideyuki Iwahata · Shu Hashimoto · Masayasu Inoue · Tomoko Inoue · Keijiro Ito · Yoshiharu Nakaoka · Nao Suzuki · Yoshiharu Morimoto

Received: 13 November 2014 / Accepted: 6 January 2015 / Published online: 24 January 2015

Abstract
Purpose Closed vitrification poses a risk of adversely affecting embryo development, while it may minimize the risk of contamination. We assessed the effects of closed-system human embryo vitrification on fetal development after implantation, neonatal outcome, and clinical safety.
Methods This was a retrospective cohort study conducted at a private fertility clinic. A total of 875 vitrified-warmed blastocysts that were single-transferred under hormone-replacement cycles between November 2011 and December 2013 were randomly divided into two groups (closed vitrification, n 313; open vitrification, n 562) after receiving the patients' consent forms. Developmental competence after implantation, including gestational age, birth weight, sex, Apgar score, and anomalies of newborns, after the transfer of blastocysts vitrified by closing vitrification was compared with that obtained in the case of open vitrification.
Results There were no significant differences between the use of closed and open vitrification systems in embryo development after implantation, gestational age, birth weight, sex ratio, Apgar score, and congenital anomalies of newborns.
Conclusion Human embryos can be vitrified using a closed vitrification system without impairment of neonatal development.

Keywords Closed vitrification system · Human blastocyst · Neonatal outcome

Capsule Closed vitrification, which eliminates the risk of cross-contamination during cooling and storage in liquid nitrogen, does not cause a debilitating effect on human embryo growth and development.

H. Iwahata · S. Hashimoto (✉) · M. Inoue · T. Inoue · K. Ito · Y. Nakaoka · Y. Morimoto
IVF Namba Clinic, 1-17-28 Minami-horie, Nishi-ku, Osaka 550-0015, Japan
e-mail: hashimoto@ivfnamba.com

H. Iwahata · S. Hashimoto · N. Suzuki
Department of Obstetrics and Gynecology, St. Marianna University School of Medicine, Kanagawa, Japan

Introduction

Since achievement of the first successful pregnancy after the transfer of a frozen human embryo [1], embryo cryopreservation has greatly contributed to the progress of human-assisted reproductive technology (ART), including the prevention of ovarian hyperstimulation syndrome and efficient use of surplus embryos. Further developments in embryo cryopreservation have been achieved through the use of ultra-rapid vitrification, which was originally applied to murine embryos by Rall and Fahy [2].

The most important consideration of the vitrification procedure is to minimize the possibility of extra- and intracellular formation of ice crystals, which would impair organelles and cell membranes during the cooling and warming phases [3–5]. Toward this end, cooling and warming rates are generally maximized by using the smallest volume possible of cryoprotectant medium to surround the cells, and the specimens are then exposed directly to liquid nitrogen without any thermo-insulation. This procedure is known as an open vitrification system. This idea was initially proposed for freezing *Drosophila* embryos [6], and drastic improvement in viability has since been shown in both animal studies [7–10] and clinical reports [11, 12]. However, there are some potential drawbacks of the open vitrification system, such as the sterility of liquid nitrogen and the risk of cross-contamination during long-term storage [13, 14]. Such cross-contamination could arise from direct contact of the solution containing the oocytes and embryos with the liquid nitrogen.

Therefore, to avoid the possible risk of contamination, closed vitrification systems have been developed [15–25]. However, new concerns such as a potential rise in temperature caused by a heat sealer and a decrease in the cooling rate have emerged with these methods. In addition, the recovery rate of embryos after warming has been shown to be lower with the use of one type of closed vitrification system compared to an open vitrification system [20].

We recently reported no significant difference between the use of closed and open vitrification systems in the survival rate, blastulation rate, proportion of good blastocysts, mean number of cells, or implantation rate [24]. In this closed system, an embryo is inserted into a straw with super-cooled air for vitrification, and its open end is sealed using ultrasound adhesion to avoid the risks of increased temperature and contamination. Subsequently, the vitrified embryos are warmed by direct exposure to a warming solution. Therefore, these embryos are vitrified, cryopreserved, and warmed without direct exposure to liquid nitrogen. Another research group also revealed no significant difference between the use of closed and open vitrification systems in the survival rate or implantation rate [25]. Recently, a total of 114 infants were obtained from blastocysts vitrified using the same closed vitrification device [25] and another closed device [26]. However, despite this apparent success, the perinatal outcomes of embryos vitrified using the closed system following implantation remains unknown. To the best of our knowledge, there are only two reports comparing neonatal data between closed and open vitrification systems [25, 26]. However, these analyses combined data of single- and multiple-embryo transfers. Since multiple pregnancies increase the risk of complications at birth, i.e.,: the risk of extreme preterm birth (28 weeks) is increased 3-fold for twins and 13-fold for triplets, and the risk of very preterm birth (28–32 weeks) is increased by almost 5-fold for twins and 20-fold for triplets [27], it is difficult to accurately and independently assess the effect of vitrification protocols (closed vs. open) on the neonatal outcome from these data.

In the present work, we compared the neonatal outcome and clinical safety using the closed vitrification system in comparison with an open vitrification system after single blastocyst transfer.

Materials and methods

This was a retrospective cohort study that was approved by the ethics committee of the IVF Namba Clinic. The data pertaining to a total of 875 vitrified-warmed blastocysts that were single-transferred under hormone-replacement cycles between November 2011 and December 2013 were randomly divided into two groups according to the day of blastocyst vitrification (closed vitrification, n 313; open vitrification, n 562) after receiving informed consent. All embryos were obtained from stimulation cycles. Some data on the viability and implantation potential of the vitrified embryos in the present work are provided in our previous study [24].

Vitrification

The Rapid-i Kit (Vitrolife Japan; Tokyo, Japan) is a closed vitrification system containing a polymethyl methacrylate stick (Rapid-i) and a thermoplastic elastomer storage straw (RapidStraw). Rapid-i has a 50-nL loading hole designed for receiving an embryo from a pipette under microscopy [19, 24]. The Rapid-i Kit also contains a stainless steel rod inserted into RapidStraw for cooling prior to insertion of the device (a rod is removed 20–30 s before insertion of Rapid-i). Cryotop®(Kitazato Corporation; Tokyo, Japan) [15] was used as the open vitrification system.

Embryos were equilibrated in 7.5 % (*v/v*) ethylene glycol (EG, Wako Chemical; Osaka, Japan), 7.5 % (*v/v*) dimethyl sulfoxide (Sigma-Aldrich; St. Louis, MO, USA), 20 % (*v/v*) serum substitute supplement (SSS, Irvine Scientific; St. Ana, CA, USA), and TCM 199 medium (Invitrogen; Tokyo, Japan) for a maximum of 10 min; shrinkage and re-expansion were confirmed, and then the embryos were transferred to vitrification solution consisting of 15 % (*v/v*) EG, 15 % (*v/v*) dimethyl sulfoxide, 0.5 M sucrose (Wako Chemical), 20 % (*v/v*) SSS, and TCM 199 medium. Each embryo was picked up with 50 nL of vitrification solution and pipetted into a hole of Rapid-i. Then, the specimens were immediately placed in super-cooled air inside a RapidStraw dipped in liquid nitrogen. The straw was then sealed using an ultrasonic sealer as described previously [24]. The sealed straw was stored in liquid nitrogen for several weeks. For the open vitrification system, after equilibration in vitrification solution, each embryo was picked up as described for the closed vitrification system and placed on a fine polypropylene strip of the Cryotop. The strip was then immediately submerged in liquid nitrogen.

Warming

After clipping the end of the straw, the Rapid-i stick was removed and the vitrified embryos were warmed in 1 mL TCM 199 containing 20 % SSS and 1 M sucrose at 37 °C for 1 min. The specimens were diluted in TCM 199 containing 20 % SSS and 0.5 M sucrose, and then diluted twice in TCM 199 containing 20 % SSS for 5 min at room temperature. The embryos vitrified using the open system were also warmed and diluted in a similar manner.

Blastocyst quality score (BQS)

To establish a numerical blastocyst morphology grading system based on Gardner's grading system [28], the blastocyst grade was converted to the multiplicative BQS proposed by Rehman et al. [29]. The BQS is a metric of blastocyst quality that is based on established morphological criteria, and is defined as the product of the degree of expansion and hatching status and ICM and TE grades, where grade A is given the value 3, grade B is given a value of 2, and grade C is given a value of 1. For example, for a 3AB blastocyst, the BQS is $3\times 3\times 2=18$.

Preparation of the endometrium

The endometrium was prepared by administration of GnRH agonist (600 μg/day, Suprecur® nasal solution 0.15 %; Mochida Pharmaceutical; Tokyo, Japan) for 3 weeks followed by increasing doses of oral estradiol valerate (Progynova®; Bayer Schering Pharma; Zürich, Switzerland) from 1 to 4 mg for 2 weeks [30]. After ultrasonographical confirmation that the endometrium was thicker than 8 mm, chlormadinone acetate (Lutoral®; Shionogi & Co.; Osaka, Japan) was administered (6 mg/day). Progesterone (Progeston depot® 125 mg; Fuji Pharma Co.; Toyama, Japan) was administered intramuscularly on the day of embryo transfer, with two additional doses after conception. Blastocyst transfer was carried out on the 5th day of chlormadinone acetate administration. Daily doses of 3 mg estradiol valerate and 6 mg chlormadinone acetate were maintained until the time of pregnancy test. When pregnancy was confirmed, estradiol (2.88 mg every 2 days, Estradna®; Hisamitsu; Saga, Japan) and progesterone (400 mg/day, Utrogestan® 200 mg; Ferring Pharmaceuticals; West Drayton, UK) were administered transcutaneously and transvaginally, respectively, until 9 weeks of gestation.

Outcome variables

Implantation was determined at around 3 weeks after embryo transfer by the detection of a single intrauterine gestational sac by transvaginal ultrasound. Fetal heart beat was confirmed beyond 6 weeks of gestation by ultrasound. Fetal loss before 22 weeks was defined as miscarriage and that after 22 weeks was defined as stillbirth. In the case of abortion for any cause, the karyotype of the abortus was analyzed as described previously [31]. Slide preparations and G-banding of chromosomes were conducted according to standard protocols [32].

The neonatal outcomes were assessed by the mean gestational age, birth weight, sex, Apgar score (evaluated within 5 min of birth), and congenital anomalies.

Statistical analysis

Differences between pairs of groups were determined using an unpaired Student's *t*-test or a χ^2 test. *P*-values<0.05 were considered to be significant. Data are presented as mean ± SE for the *t*-tests. Statistical analysis was performed using StatView version 5 (SAS Institute Inc.; Cary, NC, USA).

Results

Embryo implantation potential of thawed embryos

The patients' characteristics are provided in Table 1. The implantation rate in the closed vitrification system group was 49.7 % (150/302), which was similar to that in the open vitrification system group (49.4 %, 266/539).

Fetal or embryo development after implantation

Table 2 shows the developmental characteristics after the implantation in the two groups. Data were calculated based on the number of implantations (closed: 150, open: 266). There were no significant differences between the two systems in the frequencies of detection of a heartbeat (closed: 89.3 % vs. open: 89.5 %), miscarriage (closed: 22.0 % vs. open: 22.2 %), stillbirth (closed: 0.7 % vs. open: 0.0 %), monozygotic twins (closed: 0.0 % vs. open: 0.8 %), and live births (closed: 75.3 % vs. open: 77.4 %). There was no difference in the chromosomal aberration rate of the abortus between embryos vitrified using the closed (40 %, n 15) and open (67.6 %, n 34) systems.

Neonatal birth characteristics

Neonatal birth characteristics are presented in Table 2. Data were calculated based on the number of live births (closed: 113, open: 206). There were no significant differences between the two systems in the proportions of live births before 32 weeks (closed: 2.7 % vs. open: 1.0 %), from 32 to 34 weeks (closed: 0.9 % vs. open: 1.9 %), from 34 to 37 weeks (closed: 3.5 % vs. open: 4.9 %), and over 42 weeks (closed: 0.9 % vs.

Table 1 Baseline patient clinical characteristics and embryo implantation potential of thawed embryos vitrified with a closed or open vitrification system in hormone-replacement cycles

	Closed vitrification	Open vitrification	*P*-value
Age (years, mean ± SE)	35.6±0.2 (*n*=313)	36.0±0.2 (*n*=561)	0.168
Proportion of ICSI cycles	61.3 % (*n*=313)	61.7 % (*n*=561)	0.923
Mean no. of previous embryo transfer	1.3±0.1 (*n*=313)	1.5±0.1 (*n*=561)	0.068
Proportion of day-5 blastocysts	76.5 % (*n*=313)	73.8 % (*n*=561)	0.397
Blastocyst quality score	19.8±0.5 (*n*=313)	19.6±0.4 (*n*=561)	0.678
Survival rate after vitrification	96.5 % (*n*=313)	96.1 % (*n*=561)	0.762
Endometrial thickness (mm)	11.0±0.1 (*n*=302)	10.9±0.1 (*n*=539)	0.138
Implantation[a]	49.7 % (*n*=302)	49.4 % (*n*=539)	0.889

[a] Implantation was determined by the detection of a single intrauterine gestational sac

Table 2 Fetal development after implantation and neonatal birth characteristics of thawed embryos vitrified with a closed or open vitrification system in hormone-replacement cycles

	Closed vitrification	Open vitrification	*P*-value
Detection of heart beat (%)	89.3 (*n*=150)	89.5 (*n*=266)	0.965
Miscarriage rate (%)	22.0 (*n*=150)	22.2 (*n*=266)	0.966
Stillbirth rate (%)	0.7 (*n*=150)	0.0 (*n*=266)	0.183
Proportion of monozygotic twins (%)	0.0 (*n*=150)	0.8 (*n*=266)	0.288
Live birth rate (%)	75.3 (*n*=150)	77.4 (*n*=266)	0.626
Missing information (%)	2.0 (*n*=150)	0.4 (*n*=266)	0.104
Mean gestational age (days, mean ± SE)	275.6±1.4 (*n*=113)	274.1±1.2 (*n*=206)	0.413
Proportion of births before 32 weeks (%)	2.7 (*n*=113)	1.0 (*n*=206)	0.248
Proportion of births from 32 to 34 weeks (%)	0.9 (*n*=113)	1.9 (*n*=206)	0.469
Proportion of births from 34 to 37 weeks (%)	3.5 (*n*=113)	4.9 (*n*=206)	0.585
Proportion of births at normal gestational age (259–293 days) (%)	92.0 (*n*=113)	89.8 (*n*=206)	0.516
Proportion of births over 42 weeks (%)	0.9 (*n*=113)	1.9 (*n*=206)	0.469
Mean birth weight (g)	3127.9 (*n*=113)	3056.8 (*n*=206)	0.227
Birth weight less than 1500 g (%)	1.8 (*n*=113)	1.5 (*n*=206)	0.826
Birth weight between 1500 and 2500 g (%)	6.3 (*n*=113)	7.3 (*n*=206)	0.723

open: 1.9 %). The proportion of normal gestational age per live birth in the closed system group (92.0 %) was also similar to that in the open system group (89.8 %). There were no statistical differences in the mean birth weight (closed: 3127.9 g vs. open: 3056.8 g), or in the proportions of neonates with a birth weight less than 1500 g (closed: 1.8 % vs. open: 1.5 %) and 1500–2500 g (closed: 6.3 % vs. open: 7.3 %) between the two systems.

Table 3 shows the neonatal data of babies born at normal gestational age. Data were calculated based on the number of babies born at normal gestational age in each group (closed: 104, open: 185). There were no significant differences in the maternal age (closed: 34.7 years vs. open: 35.0 years) and maternal body mass index (closed: 20.7 vs. open: 20.3) between women in the two groups. There were also similarities between the groups in mean gestational age (closed: 278.4 days, open: 277.1 days), birth weight (closed: 3207.5 g, open: 3125.4 g), Apgar score (closed: 9.3, open: 9.3), proportion of Caesarian sections (closed: 36.5 %, open: 40.5 %), and proportion of male babies (closed: 43.3 %, open: 48.4 %). The congenital anomalies included 1 case of aproctia, 1 cleft lip and lymphangioma, and 1 syndactylus in

Table 3 Neonatal birth characteristics at normal gestational age (259–293 days)

	Closed vitrification (*n*=104)	Open vitrification (*n*=185)	*P*-value
Age (years, mean ± SE)	34.7±0.3	35.0±0.3	0.420
Proportion of ICSI cycles (%)	65.4	62.2	0.587
Body mass index (kg/mm^2)	20.7±0.3	20.3±0.2	0.218
Proportion of Caesarian sections (%)	36.5	40.5	0.505
Proportion of male babies (%)	43.3	48.4	0.406
Mean gestational age (days)	278.4±0.9	277.1±0.7	0.263
Mean gestational age of boys (days)	277.1±1.5	277.1±1.0	0.995
Mean gestational age of girls (days)	279.5±1.2	277.3±0.9	0.151
Mean birth weight (g)	3207.5±40.6	3125.4±27.2	0.084
Mean birth weight of boys (g)	3286.2±58.3	3183.3±38.2	0.132
Mean birth weight of girls (g)	3147.5±55.3	3069.2±38.3	0.233
Proportion of body weight < 2500 g	2.9	4.9	0.420
Mean Apgar score	9.3±0.07	9.3±0.05	0.815
Proportion of congenital anomalies[a]	2.9	0.5	0.102

[a] One case of aproctia, 1 cleft lip and lymphangioma, and 1 syndactylus were observed in the closed vitrification group (3/104), and 1 hemia inguinalis was found in the open vitrification group (1/185)

the closed system group (2.8 %), and 1 case of hernia inguinalis in the open system group (0.5 %). These values were not statistically different.

Discussion

The perinatal outcomes of embryos vitrified using a closed system following implantation remain obscure because conventional research comparing neonatal data between closed and open vitrification systems [25, 26] has thus far relied on combined data of single- and multiple-embryo transfers. In fact, multiple pregnancies increase the risk of complications at birth [27]. Thus, we investigated the neonatal outcome and clinical safety using the closed vitrification system in comparison with an open vitrification system after single blastocyst transfer. The results of the present work suggested that the use of a closed vitrification system supports the full-term development of vitrified human blastocysts. The data obtained from single embryo transfer is of great value for assessing the effects of closed vitrification.

There are two steps that could cause contamination during the vitrification procedure [13, 14]. The first occurs during the rapid cooling procedure via direct contact with liquid nitrogen. The second arises during long-term preservation in liquid nitrogen. The contaminated liquid nitrogen would then cause cross-contamination. Since an embryo is vitrified in supercooled air in a closed device and then packaged in a closed straw [19], the risk of contamination from liquid nitrogen could be decreased as compared with that in an open device [13, 14, 33], in a manner similar to that of conventional freezing [33] and packaged straw vitrification [34].

However, use of a closed vitrification device could potentially decrease the viability of embryos due to a decrease in the cooling rate. Slower cooling would increase the risk of ice crystal formation in the cells [4, 6]. The average rate of cooling is about −1220 °C/min in the Rapid-i device [35], whereas that of the Cryotop (open vitrification) device is −23000 °C/min [15]. Thus, the cooling rate in the closed system is markedly slower than that in the open system. However, the viability after vitrification and neonatal data obtained using the closed system were similar to those obtained using the open system. Recently, it has been shown that thawing rate is a more critical factor for embryo viability after vitrification and warming than cooling rate [36]. Since the vitrified embryos are warmed directly in the same warming solution in a similar manner, it is not surprising that no significant difference was found between two systems in the present study.

The fetal malformation rate in the closed system was 2.9 % (3/104), which was slightly higher than that in the open system (0.5 %), despite the lack of statistical significance. However, this value is similar to those obtained in a European large prospective study (3.38 %: 96/2840 in ICSI and 3.79 %: 112/2955 in in vitro fertilization), a US retrospective cohort study (5.4 %: 21/392), and in our previous study (2.3 %: 19/829) [37–39].

According to the latest available data, over 104,000 cryopreserved embryo replacements were performed in Europe in 2010 and more than 15,600 deliveries were reported as a result of cryopreserved embryo transfer [27]. Thus, the closed system, which enables aseptic vitrification without impairing the developmental competence of human embryos, could potentially have a large impact in ART.

Our study offers some insights into the safety of closed vitrification. Although there were no significant differences in the developmental characteristics after implantation or in the neonatal status between the closed and open vitrification groups, further study will be required to assess the subsequent growth and development of the children.

Acknowledgments This work was supported in part by a grant from the Japan Society for the Promotion of Science (JPS-RFTF 23580397 to S.H.).

Conflicts of interest None of the authors has a conflict of interest to disclose. Some of these data were presented at the 69th Annual Meeting of the American Society for Reproductive Medicine, October 12–17, 2013 in Boston, and a part of the data of viability after vitrification was reported in J Assist Reprod Genet 2013; 30:371–376.

References

1. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983;305:707–9.
2. Rall W, Fahy G. Ice-free cryopreservation of mouse embryos at −196 °C by vitrification. Nature. 1985;313:573–5.
3. Liebermann J, Tucker MJ. Effect of carrier system on the yield of human oocytes and embryos as assessed by survival and developmental potential after vitrification. Reproduction. 2002;124:483–9.
4. Mazur P, Seki S, Pinn IL, Kleinhans FW, Edashige K. Extra- and intracellular ice formation in mouse oocytes. Cryobiology. 2005;51:29–53.
5. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online. 2006;12:779–96.
6. Mazur P, Cole KW, Hall WH, Schreuders PD, Mahowald AP. Cryobiological preservation of Drosophila embryos. Science. 1992;258:1932–5.
7. Huang JY, Chen HY, Tan SL, Chian RC. Effect of choline-supplemented sodium-depleted slow freezing versus vitrification on mouse oocyte meiotic spindles and chromosome abnormalities. Fertil Steril. 2007;88:1093–100.
8. Lane M, Gardner DK. Vitrification of mouse oocytes using a nylon loop. Mol Reprod Dev. 2001;58:342–7.
9. Nedambale TL, Dinnyes A, Groen W, Dobrinsky JR, Tian XC, Yang X. Comparison on in vitro fertilized bovine embryos cultured in KSOM or SOF and cryopreserved by slow freezing or vitrification. Theriogenology. 2004;62:437–49.

10. Valojerdi MR, Salehnia M. Developmental potential and ultrastructural injuries of metaphase II (MII) mouse oocytes after slow freezing or vitrification. J Assist Reprod Genet. 2005;22:119–27.
11. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril. 2008;90:186–93.
12. Martinez-Burgos M, Herrero L, Megias D, Salvanes R, Montoya MC, Cobo AC, et al. Vitrification versus slow freezing of oocytes: effects on morphologic appearance, meiotic spindle configuration, and DNA damage. Fertil Steril. 2011;95:374–7.
13. Bielanski A. The potential for animal and human germplasm contamination through assisted reproductive technologies. Trends Reprod Biol. 2006;2:13–36.
14. Bielanski A, Vajta G. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum Reprod. 2009;24:2457–67.
15. Kuwayama M, Vajta G, Leda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod Biomed Online. 2005;11: 608–14.
16. Isachenko V, Montag M, Isachenko E, Zaeva V, Krivokharchenko I, Shafei R, et al. Aseptic technology of vitrification of human pronuclear oocytes using open-pulled straws. Hum Reprod. 2005;20:492–6.
17. Isachenko V, Katkov II, Yakovenko S, Lulat AG, Ulug M, Arvas A, et al. Vitrification of human laser treated blastocysts within cut standard straws (CSS): novel aseptic packaging and reduced concentrations of cryoprotectants. Cryobiology. 2007;54:305–9.
18. Vanderzwalmen P, Ectors F, Grobet L, Prapas Y, Panagiotidis Y, Vanderzwalmen S, et al. Aseptic vitrification of blastocysts from infertile patients, egg donors and after IVM. Reprod Biomed Online. 2009;19:700–7.
19. Larman MG, Gardner DK. Vitrification of mouse embryos with super-cooled air. Fertil Steril. 2011;95:1462–6.
20. AbdelHafez F, Xu J, Goldberg J, Desai N. Vitrification in open and closed carriers at different cell stages: assessment of embryo survival, development. DNA integrity and stability during vapor phase storage for transport. BMC Biotechnol. 2011;11:29.
21. Panagiotidis Y, Kasapi E, Goudakou M, Papatheodorou A, Pasadaki T, Petousis S, et al. Open vs. closed vitrification system for the cryopreservation of human blastocysts: a prospective randomized study. Hum Reprod. 2012;27:i59–60.
22. Papatheodourou A, Vanderzwalmen P, Panagiotidis Y, Kasapi L, Goudakou M, Pasadaki T, et al. Open versus closed oocyte vitrification system: a prospective randomized sibling-oocyte study. Hum Reprod. 2012;27:i72.
23. De Munck N, Verheyen G, Stoop D, Van Landuyt L, Van de Velde H. Survival and post-warming in vitro competence of human oocytes after high-security closed system vitrification. Hum Reprod. 2012;27:i72–3.
24. Hashimoto S, Amo A, Hama S, Ohsumi K, Nakaoka Y, Morimoto Y. A closed system supports the developmental competence of human embryos after vitrification. J Assist Reprod Genet. 2013;30:371–6.
25. 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.
26. 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.
27. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, et al. The European IVF-monitoring (EIM) and consortium, for the European society of human reproduction and embryology (ESHRE). Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. Hum Reprod. 2014;29:2099–113.
28. Gardner DK, Lane M. Culture and selection of viable human blastocysts: a feasible proposition for human IVF. Hum Reprod Update. 1997;3:367–82.
29. Rehman KS, Bukulmez O, Langley M, Carr BR, Nackley AC, Doody KM, et al. Late stages of embryo progression are a much better predictor of clinical pregnancy than early cleavage in intracytoplasmic sperm injection and in vitro fertilization cycles with blastocyst-stage transfer. Fertil Steril. 2007;87:1041–52.
30. Hashimoto S, Amo A, Hama S, Ito K, Nakaoka Y, Morimoto Y. Growth retardation in human blastocysts increases the incidence of abnormal spindles and decreases implantation potential after vitrification. Hum Reprod. 2013;28:1528–35.
31. Hashimoto S, Nishihara T, Murata Y, Oku H, Nakaoka Y, Fukuda A, et al. Medium without ammonium accumulation supports the developmental competence of human embryos. J Reprod Dev. 2008;54: 370–4.
32. Rooney DE, Czepulkowski BH. Human cytogenetics. A practical approach. New York: Oxford University Press; 1992.
33. 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.
34. Yokota Y, Sato S, Yokota M, Ishikawa Y, Makita M, Asada T, et al. Successful pregnancy following blastocyst vitrification: case report. Hum Reprod. 2000;15:1802–3.
35. CryoBio: pre-market notification K092398 HSV straw. 2010.
36. 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;59:75–82.
37. Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P, et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). Hum Reprod. 2002;19:671–94.
38. Budinetz TH, Mann JS, Griffin DW, Benadiva CA, Nulsen JC, Engmann LC. Maternal and neonatal outcomes after gonadotropin-releasing hormone agonist trigger for final oocyte maturation in patients undergoing in vitro fertilization. Fertil Steril. 2014;102:753–8.
39. Koike A, Nakaoka Y, Tarui S, Ohgaki A, Sugihara K, Nagata F, et al. Analysis of clinical outcomes from pregnancies achieved by frozen-thawed embryo transfer. J Fertil Implant. 2008;25:219–22.