# Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program

*Krinos M. Trokoudes, M.D.,*[a] *Constantinos Pavlides, M.Sc.,*[a] *and Xiao Zhang, M.D., Ph.D.*[a,b]

[a] Pedieos IVF Center, Nicosia, Cyprus; and [b] Cork Fertility Centre, Cork, Ireland

**Objective:** To compare the survival, fertilization, early embryonic development, and clinical outcomes from fresh and vitrified cohort oocytes.
**Design:** Review of egg-sharing program, in which the eggs from one donor were shared by recipients of fresh and vitrified eggs.
**Setting:** IVF center.
**Patient(s):** 77 women, comprising 36 patients receiving vitrified donor oocytes and 41 patients receiving fresh donor oocytes.
**Intervention(s):** Shared donor eggs vitrified by the Cryotop method warmed after vitrification, with microinjection of surviving metaphase 2 (MII) or fresh oocytes.
**Main Outcome Measure(s):** Survival, fertilization, cleavage rate, pregnancy rate, and implantation.
**Result(s):** Of the vitrified oocytes, 192 (91.4%) survived. There was no statistically significant difference in fertilization and cleavage rates, embryo quality, or clinical results obtained from vitrified compared with fresh oocytes. The outcomes of cycles using fresh oocytes were predictive of the cycle outcomes after warming of oocytes from the same cohort.
**Conclusion(s):** Oocyte donations using vitrified oocytes can provide the same quality of embryos, pregnancy, and implantation potential as fresh oocyte donations. (Fertil Steril® 2011;95:1996–2000. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Cryopreservation, IVF, oocyte donation, oocyte vitrification

An increasing number of women in developed countries are not having offspring in the early stages of their reproductive life for a variety of social and medical reasons. However, women of more advanced in age suffer from impaired fertility related to their decreasing ovarian reserve (1). A consequence has been a stronger demand for oocyte donation, but it has been accompanied by a shortage of oocyte donors (2). The shortage of oocyte donors in most countries in the world has become a major problem for both recipients and fertility specialists. In the western world, for a variety of religious, ethical, regulatory, financial, and other reasons, oocyte donation is not widely available. As a result, cross-border reproductive care has emerged, whereby couples or individuals travel to other countries to receive the treatment that they cannot get at home. Fresh oocyte donation is a proven in vitro fertilization (IVF) technology, but it is still restricted by several difficulties, such as donor availability, cost, the need to synchronize donor and recipient schedules, travel requirements, and the inability to quarantine oocytes. Oocyte cryopreservation could be a promising solution for fertility preservation and donor oocyte banking (3).

Vitrification, a new method of cryopreservation, has been reported to be a simple, cost-effective, efficient method for cryopreservation of mammalian and human oocytes as well as embryos at the cleavage, morula, and blastocyst stage (4–8). Vitrification is achieved by combining a high concentration of cryoprotectants with high cooling and warming rates. In theory, crystal formation, which is considered the main cause of cryopreservation injury, can be completely avoided by use of vitrification (9, 10).

Although there is a growing body of evidence supporting oocyte vitrification, prospective and well-controlled studies are still needed to investigate the efficiency, reliability, and safety of oocyte vitrification in donation programs and for future banking (11–15). In the clinical practice of oocyte donation, all oocytes have a relatively uniform quality because they are obtained from a young, homogeneous population of donors. In addition, cohort oocytes may be shared among recipients, resulting in a well-controlled model for studying the influence of vitrification on oocyte potential.

In this study, the Cryotop method for vitrification was used in the oocyte-sharing program. The fertilization, cleavage, embryo quality, and clinical outcome of both the vitrified and fresh cohort counterparts were evaluated. This paired-study critically assessed the reliability of the Cryotop method and elucidated the potential of this methodology in oocyte banking.

## MATERIALS AND METHODS

### Recipients

Our study included recipient patients attending the egg-sharing program at Pedieos IVF Center between 2007 and 2009. The etiology of the women's infertility was ovarian deficiency with or without previous recurrent IVF failure. Patients with normal semen parameters, as defined by World Health Organization (WHO) criteria, were included in this study, with intracytoplasmic sperm injection (ICSI) treatment as the method of insemination. Embryo transfers were performed on day 3. Donated oocytes from each donor were shared by one or two fresh-egg recipient(s) and one vitrified-egg recipient. The vitrified-egg recipient acquired the oocytes 1 to 3 months after the vitrification step. In total, this study involved 36 patients receiving vitrified oocytes and 41 patients receiving fresh oocytes.

Received July 24, 2010; revised February 14, 2011; accepted February 15, 2011; published online March 15, 2011.
K.M.T. has nothing to disclose. C.P. has nothing to disclose. X.Z. has nothing to disclose.
Reprint requests: Xiao Zhang, M.D., Ph.D., Cork Fertility Centre, Fernhurst House, College Road, Cork, Ireland (E-mail: zhangxiaoivf@gmail.com).



0015-0282/$36.00
doi:10.1016/j.fertnstert.2011.02.035

## Oocyte Donors

The age of the donors taking part in this study ranged between 22 and 35 years. The treatment employed was the long protocol with down-regulation starting in the midluteal phase by use of daily subcutaneous doses of a gonadotropin-releasing hormone (GnRH) agonist, buserelin. After pituitary suppression was confirmed, ovarian stimulation was achieved by using follicle-stimulating hormone (FSH) injections (Fostimon; Institut Biochimique SA [IBSA], Lugano, Switzerland); the dosage administered was tailored to each donor in a highly individualized regimen that ranged between 75 and 225 IU/day. Stimulation was monitored by ultrasound scanning and serum estradiol testing. When at least two leading follicles reached 18 mm in diameter, human chorionic gonadotropin (Choriomon; IBSA) was administered, and egg collection was performed 34 to 36 hours later.

## Recipient Preparation

All oocyte recipients received hormone replacement therapy. Estradiol valerate (Progynova; Schering AG, Berlin, Germany) was initiated on day 2 to 3 of the cycle as follows: 2 mg three times per day, supplemented by 50–100 $\mu$g estradiol transdermal patches (Evorel; Janssen-Cilag, Ltd., High Wycombe, Buckinghamshire, United Kingdom) changed every 48 hours, depending on the endometrial response. Transvaginal ultrasound, and serum estradiol and progesterone levels were used for monitoring. The recipients were started on vaginal progesterone, 200 mg three times per day (Utrogestan; Ferring Pharmaceuticals, Ltd., Langley, Berkshire, United Kingdom), from the day of the donor's oocyte collection (day 0) and intramuscular progesterone, 50 mg per day, from day 3 after insemination. The estradiol and progesterone treatment was continued until week 12 of gestation.

## Embryo Culture

The embryos were cultured in culture medium drops covered with mineral oil (Ferticult; Beernem, Belgium) in Falcon tissue culture dishes (353001; Becton Dickinson, Franklin Lakes, NJ). Specifically, 4 hours after oocyte retrieval, the oocytes were inseminated by means of ICSI and cultured in Universal IVF medium (0.1 mL; Medicult, Jyllinge, Denmark) at 37°C in an atmosphere of 5% $CO_2$. At 15 to 18 hours after insemination, fertilization was checked, and the embryos were subsequently cultured in ISM 1 medium (0.1 mL; Medicult) until day 3. Embryo transfers and, when criteria were met, cryopreservation of supernumerary embryos were performed on day 3 after fertilization.

## Oocyte Freezing and Warming

The Cryotop method for oocyte vitrification was used as previously described by Kuwayama et al. (11). Oocytes were equilibrated in 7.5% (v/v) ethylene glycol (EG) + 7.5% dimethylsulfoxide (DMSO) in TCM199 medium + 20% synthetic serum substitute (SSS), referred to as equilibrium solution, at room temperature for 10 to 15 minutes, depending how soon the oocytes returned to their original size from the dehydration caused by the cryoprotectants at the beginning of this step. The oocytes then were placed into vitrification solution (VS) containing 15% EG + 15% DMSO + 0.5 M sucrose. After 1 minute in this solution, oocytes were loaded on the Cryotop strip (11), and then were immediately submerged into liquid nitrogen.

For warming, the Cryotop was removed from the liquid nitrogen and instantly immersed into 1.0 M sucrose in TCM199 + 20% SSS at 37°C. After 1 minute, the oocytes were placed in 0.5 M sucrose in TCM199 + 20% SSS at room temperature for 3 minutes. Finally, two 5-minute washes were performed with TCM199 + 20% SSS at room temperature before incubating the oocytes in Universal IVF medium for 2 hours before ICSI. Supplies and solutions for vitrification were obtained from Kitazato (Tokyo, Japan).

## Assessment of Embryos

Embryo quality was assessed according to the number and form of blastomeres and the percentage of cytoplasmic fragmentation, as reported by Plachot and Mandelbaum (16). For statistical comparison purposes and to objectively quantify embryo quality, these three variables were scored according to fixed criteria and then assigned an arbitrary score of 0 to 4.

**TABLE 1**

**Characteristics of recipients of fresh and vitrified donor oocytes.**

| Characteristics | Fresh oocyte | Vitrified oocyte | *P* value |
|---|---|---|---|
| No. of recipients | 41 | 36 | N/A |
| Age of recipient (y) | 44.4 ± 0.92 | 45.4 ± 0.87 | .24 |
| Age of recipient's partner (y) | 46.7 ± 0.92 | 47.3 ± 0.99 | .49 |
| No. of oocytes (MII) | 247 | 210 | N/A |
| Mean no. of oocytes received | 6.0 ± 0.24 | 5.8 ± 0.21 | .11 |

*Note:* MII = metaphase II; N/A = not applicable.

*Trokoudes. Vitrified oocytes for donation. Fertil Steril 2011.*

Embryos with fewer than four blastomeres on day 3 after ICSI were scored with a grade 4.

In contrast, ≥6-cell embryos and four- to five-cell embryos were scored 0 and 2, respectively. Symmetrical cells were scored 0, whereas asymmetrical cells were scored 1. Embryos having <15%,15% to 30%, 30% to 50%, and ≥50% fragmentation were scored 0, 1, 2, and 3, respectively. Accordingly, an optimal quality embryo would score 0. Embryos on day 3 with ≥6 cells and <30% fragmentation were regarded as good embryos (17).

## Statistical Analysis

The chi-square test was used for quantitative variables. $P<.05$ was considered statistically significant for evaluation of vitrification effects on embryo quality. The correlations between the average embryo morphology score from the vitrified oocytes and that from the fresh cohorts were estimated by Pearson's correlation analysis using SPSS 13.0 (IBM/SPSS, Inc., Chicago, IL). In addition, a paired *t*-test was employed to compare the embryo morphology score from the vitrified and the cohort fresh oocytes from the same donor.

# RESULTS

All the donors and recipients were thoroughly informed of the study details and consented. The oocyte donation and vitrification services in Pedieos IVF Center have been inspected to comply with the European Union Tissue and Cells Directive (EUTCD). From June 2007 to October 2009, 77 recipients were allocated into 36 egg-sharing pairs: 31 pairs had one vitrified-egg and one fresh-egg recipient from one donor, and 5 pairs had one vitrified-egg and two fresh-egg recipients from one donor. In total, 513 oocytes were retrieved from 36 donors, aged 29.3 ± 3.63 years; of these, 457 oocytes were identified as metaphase II (MII) due to the presence of one polar body (Table 1).

After vitrification and warming, a total of 192 of 210 (91.4%) oocytes survived. Embryo development is detailed in Table 2. Briefly, there was no difference in fertilization rates (162 [84.4%] vs. 214 [86.6%]) or day-2 cleavage (154 [95.1%] vs. 205 [95.8%]) for vitrified and fresh oocytes. In addition, the embryo quality on day 3 was similar when comparing the day-3 good embryo rate between the vitrification and fresh groups (124 [60.4%] vs. 100 [64.9%]).

A total of 36 cycles from vitrified oocytes and 41 cycles from fresh oocytes were transferred. The clinical pregnancy rate, implantation rate, ongoing pregnancy rate, and live-birth rate in vitrified and fresh oocyte groups were 55.6% versus 48.8%, 24.7% versus 25.6%, 47.2% versus 43.9%, and 41.5% versus 47.2%, respectively, with no statistically significant differences. Regarding the efficiency

**TABLE 2**

**Cycle outcomes: comparative data for recipients of fresh and vitrified oocytes.**

| Outcomes | Fresh | Vitrified | *P* value |
|---|---|---|---|
| Oocyte survival (%) | N/A | 192 (91.4%) | N/A |
| Fertilization rate (%) | 214 (86.6%) | 162 (84.4%) | .50 |
| No. of fertilized oocytes per recipient ± SD | 5.2 ± 0.26 | 4.5 ± 0.25 | .23 |
| No. of cleaved embryos per recipient ± SD | 5 ± 0.27 | 4.3 ± 0.23 | .08 |
| Good embryo on day 3 (%) | 124 (60.4%) | 100 (64.9%) | .39 |
| Embryos transferred per recipient ± SD | 2.09 ± 0.08 | 2.25 ± 0.09 | .23 |
| Embryos cryopreserved per recipient | 8 (19.5%) | 5 (13.9%) | .51 |
| Clinical pregnancy rate per embryo transfer (%) | 20/41 (48.8%) | 20/36 (55.6%) | .55 |
| Implantation rate (%) | 22/86 (25.6%) | 20/81 (24.7%) | .9 |
| Ongoing pregnancy rate per embryo transfer (%) | 18/41 (43.9%) | 17/36 (47.2%) | .89 |
| Live–birth rate (%) | 17/41 (41.5%) | 17/36 (47.2%) | .61 |

*Note:* N/A = not applicable; SD = standard deviation.

*Trokoudes. Vitrified oocytes for donation. Fertil Steril 2011.*

of oocyte vitrification, every 10.5 vitrified versus 11.2 fresh oocytes produced one clinical pregnancy (see Table 2).

It is interesting that, when comparing the outcome of vitrification cycles whose fresh counterparts achieved live birth with the vitrification cycles whose fresh counterparts did not, the former had a statistically significantly higher live-birth rate, 14 (82.4%) versus 3 (18.8%) (Fig. 1). In the consistency study of the embryo morphology of the fresh oocytes versus the vitrification counterpart cohorts, Pearson's correlation analysis revealed that there was a strong correlation between the fresh and vitrified groups (R=0.64, $P<.001$) (Fig. 2). Furthermore, there was no statistically significant difference in embryo morphology between the vitrified (2.77 ± 0.67) and fresh (2.69 ± 0.75, $P=.41$) counterparts.

## DISCUSSION

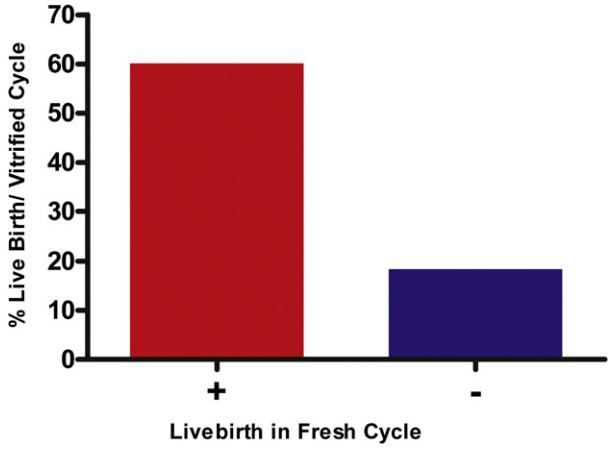
Oocyte donation is the best treatment option for women who have decreasing ovarian reserves from a variety of causes. Unfortunately, the universal shortage of oocyte donors has become a major obstacle for both patients and fertility specialists. The major difficulty in recruitment of donors is that donors are intimidated by the prospect of multiple injections, and they become frustrated by the prospect of multiple clinic visits. However, the main issue is the invasive procedure they must undergo during egg retrieval. In Europe, for a variety of religious, ethical, regulatory, financial, and other reasons, oocyte donation is not widely available in most member states. As a result, cross-border reproductive care has developed, whereby couples or individuals travel to other countries to receive treatment that they cannot get at home. Some countries, such as Spain, the Czech Republic, and Cyprus, end up attracting many medical tourists for oocyte donation due to their short waiting lists, lower costs, and favorable legislation (18).

Although fresh oocyte donation is a proven IVF technology, it is still restricted by several problems, such as donor availability, cost, and the need to synchronize donor and recipient schedules, and travel requirements as well as the inability to quarantine oocytes. The use of frozen donor eggs alleviates some of these difficulties, such as the coordination between donor and recipient cycles and precise traveling times. An added advantage is that banks of cryopreserved donated oocytes can make egg donation safer by adopting a quarantine policy similar to that employed by sperm banks (19).

Cryopreservation of sperm and embryos has been an integral part of infertility treatment for some time (20); As a result, assisted reproduction treatments have become more flexible and efficient. Freezing the MII oocyte, however, has met with difficulties due to its large size, water content, and chromosomal arrangement. In the mature oocyte, the metaphase chromosomes are lined up by the meiotic spindle along the equatorial plate and are easily damaged during the slow-freezing/thawing process (21, 22). It has been more than two decades since the first successful report (23) of oocyte cryopreservation using this method. Although additional reports of successful oocyte freezing and normal pregnancies followed, the method did not become a routine IVF procedure because of its inefficiency: usually 23 to 94 oocytes were needed for one successful pregnancy (24–28). In contrast, vitrification has been reported more recently as an alternative cryopreservation method that is characterized by simplicity, low cost, and high efficiency for cryopreserving both oocytes and embryos (4–8). Specifically, the Cryotop system, featuring a minimum loading amount, has been reported to be very successful in oocyte cryopreservation (11, 12, 29).

**FIGURE 1**

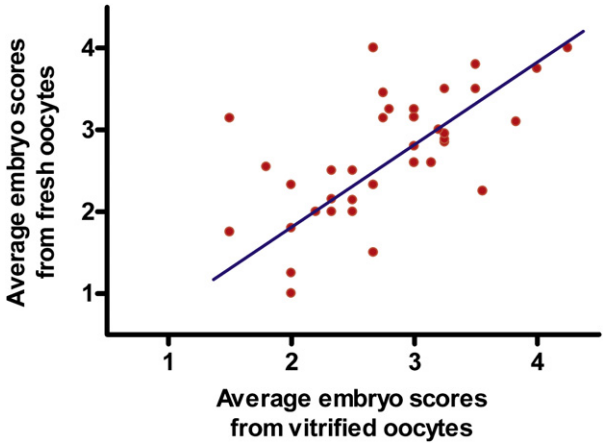
Outcome of vitrification cycles in which fresh counterparts achieved live birth (+) versus vitrification cycles in which fresh counterparts did not (–). There was a statistically significant difference in live birth rates (60.0% vs. 18.2%, $P<.05$).




*Trokoudes. Vitrified oocytes for donation. Fertil Steril 2011.*

## FIGURE 2

Pearson's correlation between the average embryo score of vitrified and fresh oocyte recipients from the same oocyte donor (R=0.639, $P<.001$).




*Trokoudes. Vitrified oocytes for donation. Fertil Steril 2011.*

Our study has confirmed earlier reports that vitrification can be achieved by fast cooling and minimum liquid volume. We implemented the Cryotop system in our oocyte donation program. In total, 192 of 210 (91.4%) oocytes survived after vitrification. We found that there was no statistically significant difference in fertilization rates (84.4% vs. 86.6%) between the vitrified and fresh oocytes, which is similar to what other researchers have obtained with other vitrification systems (13, 30–35). This suggests that vitrified oocytes retain their morphologic and functional integrity. To further assess the influence of the vitrification procedure on embryo development, we observed similar rates of cleavage on day 2 (95.1% vs. 95.8%) and good embryo development (60.4% vs. 64.9%) on day 3 after insemination in both vitrified and fresh oocytes, respectively. Most importantly, vitrified oocytes have had similar implantation potential to that of fresh oocytes. The data from this study show that the pregnancy rate per vitrified oocyte and implantation rate are similar to that of fresh oocytes, which is supported by other investigations using the Cryotop method (11–13). The consistent efficiency of oocyte vitrification justifies its use as a routine method for oocyte donation.

Early embryonic development depends greatly on the final stages of oocyte development, during which there is an intensive accumulation of cytoplasmic organelles, proteins, and RNA in the oocyte cytoplasm (36). The type of ovarian stimulation implemented in the donor cycles has a major effect on embryo quality and clinical outcomes (37–40). Consequently, oocyte quality varies among individuals as well as among cycles.

Several studies that have focused on vitrified oocyte donation have failed to directly compare their results with well-controlled fresh cycles or have provided only embryology data (13). So from this point of view, an oocyte-share paired study offers the best model to study the impact of vitrification on oocytes. In addition, to minimize the effect of sperm on embryo quality, only couples with normal semen parameters were included in our study, and inseminations were all performed by ICSI. We compared sibling embryos in a recipient pair, which provided direct information of how vitrification impacts on oocytes. In our study, embryos from vitrified oocytes showed no difference in morphology score on day 3 when compared with their fresh cohorts ($2.77 \pm 0.67$ vs. $2.69 \pm 0.75$). Pearson's correlation analysis revealed a strong correlation of embryo morphology between the fresh and vitrified groups (see Fig. 2). This means that the quality of vitrified oocytes depends on the nature of the oocyte and is not affected by the vitrification process itself.

Furthermore, consistency in embryo morphology was manifested in clinical outcomes. Vitrified oocytes produced higher live birth rates when live birth was obtained from their cohort fresh oocytes (see Fig. 1). This implies that the developmental potential of vitrified oocytes can be predicted by the results from their cohort's fresh oocytes. This finding may be used as an important parameter in the quality assessment of vitrified oocytes in oocyte banking systems.

There is, however, one issue with vitrification that needs further discussion. The vitrification system used in this study is based on minimized loading solution and an ultracooling procedure. With the Cryotop, the embryos were in direct contact with liquid nitrogen. During the vitrification procedure, there have been concerns about the possible risk of cross-contamination via liquid nitrogen contact (41, 42), especially for biopsied embryos, which have inconsistent zonae. One possible solution is filtering the liquid nitrogen with a 0.2-$\mu$m filter to eliminate bacteria and fungi and storing it in a vapor phase of liquid nitrogen (VPLN) tank (43) to minimize the risk of contamination. The other is the use of a closed system, in which oocytes or embryos are sealed in straws and contact the liquid nitrogen indirectly. Recently, promising clinical reports with high security straws have shown that the closed system has the same efficiency (44, 45).

To our knowledge, our study has shown for the first time consistent embryology and clinical results of cryopreserved and fresh cohort oocytes. These results thus may form the basis of efficiency assessment of oocyte vitrification during the setup of oocyte banks. When the embryological and clinical outcome of vitrified versus fresh cohort oocytes in paired patients undergoing oocyte donation were compared, the results showed that oocyte vitrification based on the Cryotop system had no adverse effects on the oocyte development during donation. Vitrification can provide the same quality of embryo, pregnancy, and implantation potential as fresh oocyte donation and therefore can be adopted as a routine oocyte donation and cryobanking solution.

*Acknowledgments:* The authors thank Dr. Tim Dineen and Dr. Michelle O'Connor, members of Cork Fertility Centre, for their assistance in the data analysis and the preparation of the manuscript.

## REFERENCES

1. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992;7:1342–6.
2. Thum MY, Gafar A, Wren M, Faris R, Ogunyemi B, Korea L, et al. Does egg-sharing compromise the chance of donors or recipients achieving a live birth? Hum Reprod 2003;18:2363–7.
3. 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;92:520–6.

4. Kuwayama M, Vajta G, Ieda 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.
5. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology 2007;67:73–80.
6. Desai N, Blackmon H, Szeptycki J, Goldfarb J. Cryoloop vitrification of human day 3 cleavage-stage embryos: post-vitrification development, pregnancy outcomes and live births. Reprod Biomed Online 2007;14:208–13.
7. Al-Hasani S, Ozmen B, Koutlaki N, Schoepper B, Diedrich K, Schultze-Mosgau A. Three years of routine vitrification of human zygotes: is it still fair to advocate slow-rate freezing? Reprod Biomed Online 2007;14:288–93.
8. Liebermann J, Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. Fertil Steril 2006;86:20–6.
9. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at –196 degrees C by vitrification. Nature 1985;313:573–5.
10. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril 2006;86:70–80.
11. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online 2005;11:300–8.
12. Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reprod Biomed Online 2007;14:72–9.
13. Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril 2008;89:1657–64.
14. Yoon TK, Lee DR, Cha SK, Chung HM, Lee WS, Cha KY. Survival rate of human oocytes and pregnancy outcome after vitrification using slush nitrogen in assisted reproductive technologies. Fertil Steril 2007;88:952–6.
15. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh "versus" vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod 2010;25:66–73.
16. Plachot M, Mandelbaum J. Oocyte maturation, fertilization and embryonic growth in vitro. Br Med Bull 1990;46:675–94.
17. Rienzi L, Ubaldi F, Iacobelli M, Ferrero S, Minasi MG, Martinez F, et al. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. Hum Reprod 2002;17:1852–5.
18. Treatment Abroad medical tourism Web site, at http://www.treamentabroad.com. Accessed: April 5 2010.
19. Jain JK, Paulson RJ. Oocyte cryopreservation. Fertil Steril 2006;86:1037–46.
20. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983;305:707–9.
21. Baka SG, Toth TL, Veeck LL, Jones HW Jr, Muasher SJ, Lanzendorf SE. Evaluation of the spindle apparatus of in-vitro matured human oocytes following cryopreservation. Hum Reprod 1995;10:1816–20.
22. Shaw JM, Oranratnachai A, Trounson AO. Fundamental cryobiology of mammalian oocytes and ovarian tissue. Theriogenology 2000;53:59–72.
23. Chen C. Pregnancy after human oocyte cryopreservation. Lancet 1986;1:884–6.
24. Porcu E, Fabbri R, Seracchioli R, Ciotti PM, Magrini O, Flamigni C. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. Fertil Steril 1997;68:724–6.
25. Yoon TK, Chung HM, Lim JM, Han SY, Ko JJ, Cha KY. Pregnancy and delivery of healthy infants developed from vitrified oocytes in a stimulated in vitro fertilization–embryo transfer program. Fertil Steril 2000;74:180–1.
26. Chen SU, Lien YR, Chen HF, Chang LJ, Tsai YY, Yang YS. Observational clinical follow-up of oocyte cryopreservation using a slow-freezing method with 1,2-propanediol plus sucrose followed by ICSI. Hum Reprod 2005;20:1975–80.
27. Boldt J, Tidswell N, Sayers A, Kilani R, Cline D. Human oocyte cryopreservation: 5-year experience with a sodium-depleted slow freezing method. Reprod Biomed Online 2006;13:96–100.
28. Yoon TK, Kim TJ, Park SE, Hong SW, Ko JJ, Chung HM, et al. Live births after vitrification of oocytes in a stimulated in vitro fertilization–embryo transfer program. Fertil Steril 2003;79:1323–6.
29. Morato R, Izquierdo D, Paramio MT, Mogas T. Cryotops versus open-pulled straws (OPS) as carriers for the cryopreservation of bovine oocytes: effects on spindle and chromosome con and embryo development. Cryobiology 2008;57:137–41.
30. Nottola SA, Coticchio G, Sciajno R, Gambardella A, Maione M, Scaravelli G, et al. Ultrastructural markers of quality in human mature oocytes vitrified using Cryoleaf and Cryoloop. Reprod Biomed Online 2009;19(Suppl 3):17–27.
31. Keskintepe L, Agca Y, Sher G, Keskintepe M, Maassarani G. High survival rate of metaphase II human oocytes after first polar body biopsy and vitrification: determining the effect of previtrification conditions. Fertil Steril 2009;92:1706–15.
32. Cobo A, Perez S, De los Santos MJ, Zulategui J, Domingo J, Remohi J. Effect of different cryopreservation protocols on the metaphase II spindle in human oocytes. Reprod Biomed Online 2008;17:350–9.
33. Kim TJ, Laufer LR, Hong SW. Vitrification of oocytes produces high pregnancy rates when carried out in fertile women. Fertil Steril 2010;93:467–74.
34. Almodin CG, Minguetti-Camara VC, Paixao CL, Pereira PC. Embryo development and gestation using fresh and vitrified oocytes. Hum Reprod 2010;25:1192–8.
35. 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;25:1199–205.
36. Barnes FL, Sirard MA. Oocyte maturation. Semin Reprod Med 2000;18:123–31.
37. Aboulghar MA, Mansour RT, Serour GI, Ramzy AM, Amin YM. Oocyte quality in patients with severe ovarian hyperstimulation syndrome. Fertil Steril 1997;68:1017–21.
38. Mercan R, Mayer JF, Walker D, Jones S, Oehninger S, Toner JP, et al. Improved oocyte quality is obtained with follicle stimulating hormone alone than with follicle stimulating hormone/human menopausal gonadotrophin combination. Hum Reprod 1997;12:1886–9.
39. Imthurn B, Macas E, Rosselli M, Keller PJ. Nuclear maturity and oocyte morphology after stimulation with highly purified follicle stimulating hormone compared to human menopausal gonadotrophin. Hum Reprod 1996;11:2387–91.
40. Diamond MP, DeCherney AH, Hill GA, Nero F, Wentz AC. Response to repetitive cycles of ovulation induction in the same women. J In Vitro Fert Embryo Transf 1987;4:251–5.
41. Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C. Viral contamination of embryos cryopreserved in liquid nitrogen. Cryobiology 2000;40:110–6.
42. Balaban B, Yakin K, Isiklar A, Urman B. Utilization of high-security straws for embryo freezing in an in vitro fertilization program: a prospective, randomized study. Fertil Steril 2007;87:691–6.
43. Cobo A, Pérez S, Santos MJ, Pellicer A, Remoh J. Comparison between storage of vitrified oocytes by Cryotop method in liquid nitrogen vs. vapor phase of liquid nitrogen tanks. Feril Steril 2007;88 (Suppl):S91.
44. Fasano G, Fontenelle N, Vannin AS, Duez M, Biramane J, Emiliani S, et al. A randomized controlled trial comparing two vitrification freezing methods versus slow freezing for cryopreservation of human cleavage stage embryos. Hum Reprod 2010;25(Suppl 1):i11–4.
45. Sipe C, Pelts EI, Matthews IM, Sanchez SR, Brohammer R, Wagner Y, et al. After more than 3,300 transferred vitrified blastocysts: what is the verdict? Hum Reprod 2010;25(Suppl 1):i11–4.