J Assist Reprod Genet (2017) 34:417–422
DOI 10.1007/s10815-016-0841-y

TECHNOLOGICAL INNOVATIONS

Hydroxypropyl cellulose supplementation in vitrification solutions: a prospective study with donor oocytes

Miguel Gallardo[1,2] · María Hebles[1] · Beatriz Migueles[1] · Mónica Dorado[1] · Laura Aguilera[1] · Mercedes González[1] · Paloma Piqueras[1] · Alejandro Lucas[2] · Lorena Montero[1] · Pascual Sánchez-Martín[1] · Fernando Sánchez-Martín[1] · Ramón Risco[2,3]

Received: 11 April 2016 / Accepted: 8 November 2016 / Published online: 27 December 2016

Abstract
Purpose Hydroxypropyl cellulose (HPC), a polysaccharide that forms a viscous gel under low temperatures, is a promising substitute of the blood-derived macromolecules traditionally used in cryopreservation solutions. The performance of a protein-free, fully synthetic set of vitrification and warming solutions was assessed in a matched pair analysis with donor oocytes.
Methods A prospective study including 219 donor MII oocytes was carried out, comparing the laboratory outcomes of oocytes vitrified with HPC-based solutions and their fresh counterparts. The primary performance endpoint was the fertilization rate. Secondary parameters assessed were embryo quality on days 2 and 3.
Results 70/73 (95.9%) vitrified MII oocytes exhibited morphologic survival 2 h post-warming, with 49 (70.0%) presented normal fertilization, compared to 105 of 146 (71.9%) MII fresh oocytes. Similar embryo quality was observed in both groups. A total of 18 embryos implanted, out of 38 embryos transferred (47.3%), resulting in 13 newborns.

Capsule The satisfactory results of this direct comparison with fresh controls strongly support the effectiveness of HPC supplementation of vitrification solutions, confirming its role as a substitute for protein supplementation.

✉ Ramón Risco
risco@us.es

[1] Clínica Ginemed, C/ Farmacéutico Murillo Herrera n 3-5, 41010 Seville, Spain

[2] Seville Engineering School, University of Seville, Camino de los Descubrimientos s/n, 41092 Seville, Spain

[3] National Accelerators Centre, Calle Thomas Alva Edison, 7, 41092 Seville, Spain

Keywords Vitrification · Oocyte · Hydroxypropyl cellulose · Protein supplementation · Serum free

Introduction

The ability to efficiently cryopreserve oocytes and embryos by vitrification [1] has become essential in the field of human-assisted reproduction. There are two main factors which influence the efficacy of vitrification: (i) the cooling/warming rates (which are influenced by the type of vitrification device used) and (ii) the glass-forming tendency (which is determined by the composition of the solution) [2–8]. Of course, each of these two factors depends on multiple parameters [9, 10]. With current techniques, the rates of survival of embryos and oocytes to the vitrification process have reached very high levels [11–13].

Human oocyte vitrification is a routine procedure today [14]. Historically, it has presented a greater challenge than embryo vitrification for many reasons. Oocytes' low surface to volume ratio, and the low permeability coefficient of their plasma membrane, hinders the exchange between cryoprotectant agents (CPAs) and water [15, 16]. However, this issue has been overcome by the synergic combination of permeating CPAs, mainly ethylene glycol and dimethyl sulfoxide, and non-permeating CPAs, such as sucrose and trehalose, and adjusting the concentration and the exposure time to CPAs needed to achieve a successful vitrification with currently attainable cooling and warming rates [17–22].

Another component of the solutions used for vitrification and warming is the macromolecular supplementation. It increases the viscosity of the solution to enhance the glass-forming tendency, enables the in vitro manipulation required for the vitrification procedure, and prevents the attachment of the gametes and embryos to the surfaces of

pipettes, dishes, and devices used in the process [23]. Protein supplementation with human serum albumin has traditionally been employed for this task, alone or in the form of synthetic serum substitute (SSS), or dextran serum supplement (DSS), and it is still used in most commercial formulations. However, as a human-source material, it presents risks of contamination and production variability associated with it [24], so a replacement synthetic component has been sought. The international regulatory policies are also encouraging the use of solutions free of human or animal components.

The fully synthetic macromolecule hydroxypropyl cellulose (HPC) has been a successful candidate: it is a variable length polysaccharide that, with certain molecular weights, when added to a solution, can achieve very similar physical properties to the albumin-based formulations, forming a viscous gel under low temperatures. It is listed as pharmacopeia and is a common food additive and drug excipient [25]. Preliminary studies have shown promising results of HPC supplementation of vitrification solutions on murine oocytes and blastocysts in terms of survival and embryo development [26, 27]. Recently, direct comparative studies revealed the similarity of HPC and SSS-based formulations for human oocytes vitrification [25, 28].

In the present study, we use a formulation of protein-free vitrification and warming solutions for the vitrification of oocytes from an egg donation program. Laboratory outcomes of vitrified-warmed oocytes are compared against fresh oocytes from the same donor from a different stimulation cycle. This design allows us to evaluate the impact of vitrification on the developmental abilities of donor oocytes and assess the performance of an HPC-based vitrification formulation for its use in daily laboratory practice.

Materials and methods

Study design and outcome measures

A matched pair analysis within donor was performed, involving results from 219 MII oocytes from 19 oocyte donors between June 2014 and January 2015. Each recipient was assigned with two cohorts of oocytes from the same donor; one composed by six to nine fresh oocytes from a synchronized donation cycle (control group), and the other cohort composed two to five oocytes were vitrified/rewarmed oocytes from a previous cycle of the same donor (experimental group). Both groups of oocytes underwent ICSI in parallel. The primary end-point was the fertilization rate of fresh and vitrified oocytes, with secondary assessments of embryo quality performed on day 2 and day 3.

Study population

All donors included on the study complied with the regulations on ART donors, described elsewhere [29], and the following inclusion criteria: over 18 and under 30 years old, good physical and psychological health, no personal nor familiar history for hereditary diseases, normal karyotype, negative tests for sexually transmitted diseases, and without any medical or gynecological disorders. Donors were subjected to a short agonist stimulation treatment and were excluded in case of a response to stimulation <12 oocytes. All recipients for the egg donation program were offered to participate in the study, until the quota was filled. Couples with a severe male factor surgically extracted spermatozoa and very severe oligoastenozoospermia (motile sperm count <500.000/ml after preparation) were not included.

Ethical considerations

All recipients were informed thoroughly about the protocol of the study and signed an informed consent form. The study was conducted as described in the protocol, which was developed in accordance to the principles of Helsinki and the national Policy of Good Clinical Practice (ISO 14155:2011), and was approved by the internal review board of the institutional research committee.

Donor and recipient stimulation

To synchronize the donation, donors took contraceptive pills during the previous month to the stimulation. Stimulation dose was decided according to donor's BMI (150–200 daily IU of rFSH, alpha folitropin, Gonal-F, Merck). On day 5 of stimulation, analysis of the estradiol levels and ultrasound scans was performed for dose adjustment. When a follicle reached 14 mm, they started GnRH antagonist treatment with 0.25 mg Cetrotide (Cetrorelix, Merck) until at least three follicles reached 20 mm, when ovulation was triggered with 0.3 mg of triptoreline GnRH agonist (Decapeptyl; IpsenPharma).

Recipients were kept on contraceptive pills for the synchronized donation. Once discontinued, after menstruation, they started endometrial substitutive treatment with 6 mg daily of estradiol hemihydrate (Estradot, Novartis, Switzerland). On the day of the donor's ovarian puncture, they began taking progesterone vaginal or oral supplement, 600 mg daily (Utrogestan, Seid, Spain), continued until the results of the embryo transfer were known.

Ovarian puncture

A total of 19 oocyte donation cycles were included in the study. Oocyte recovery was performed by eco-guided ovarian puncture. An ultrasound transducer was placed into the

vaginal fornix to visualize the ovary and follicles, and a single lumen ovum aspiration needle (Cook Medical) was inserted in the transducer and advanced into the ovarian follicles, where oocytes were recovered by aspiration with a syringe, until all the follicles had been punctured.

Embryology procedures

Two hours post-retrieval, the fresh control group of oocytes was denuded using hyaluronidase (HYASE-10X, Vitrolife), and the experimental group of oocytes was rewarmed as described below. Both control and experimental groups underwent ICSI simultaneously, 38–40 h post-hCG in the case of fresh oocytes and 2 h post-warming in the case of vitrified oocytes. Embryos were cultured on microdrops (G-IVF, G1Plus, G2Plus, Vitrolife) under mineral oil (tissue culture oil, Sage, CooperSurgical) until the third day of development, when they were either transferred, cryopreserved, or kept on culture up to day 6, according to their quality.

Vitrification protocol

Vitrification solutions were supplemented with 0.06–0.125 mg/ml of hydroxypropyl cellulose (pharmaceutical standard, 80,000 Da average molecular weight, Sigma Aldrich) and did not contain albumin nor any other proteins. Vitrification of the experimental group oocytes was performed at laboratory room temperature (RT), in our case 23–26 ± 0.5 °C, with SafeSpeed vitrification media (SafePreservation). The vitrification kit consists of three solutions: washing solution (WS, no CPAs), equilibration solution (ES 7.5% ethylene glycol and 7.5% dimethyl sulfoxide) and vitrification solution (VS 15% ethylene glycol and 15% dimethyl sulfoxide) [30].

All solutions were exposed at least for an hour to laboratory RT. Oocytes were gradually exposed to the cryoprotective agents by placing them in a first droplet of 50 μl of WS, which was joined with the tip of the pipette to another droplet of 50 μl of ES. After 2 min, both droplets were joined with a third ES drop and allowed an additional 2 min of CPA diffusion to the primary droplet where the oocytes were located. Afterwards, oocytes were transferred to 100 μl of ES, where they remained up to 10 min, until the oocytes appeared to be fully re-expanded. Once the equilibration was complete, oocytes were transferred to a 200-μl droplet of VS and washed at least three times to eliminate any leftover ES. Then the oocytes were placed on the thin plastic strip of a Cryotop cryodevice, as described by Kuwayama et al. [31], and plunged vertically in liquid nitrogen. The amount of time the oocytes remained in VS for washing and loading purposes before plunging in liquid nitrogen was approximately 60 s.

Warming protocol

SafeSpeed warming media consists in three solutions: thawing solution (TS) (1 M sucrose), dilution solution (DS) (0.5 M sucrose), and WS (no CPAs) [30]. For at least 1 h, closed vials of DS and WS were exposed to laboratory RT, and TS vial was placed in the incubator at 37 °C. The vitrification straw containing the oocytes was transferred from a styrofoam box with liquid nitrogen to a double-well dish (Becton-Dickinson, 60 × 15 mm Falcon Center-Well Organ Culture Dish) containing 1 ml of TS at 37 °C. The liquid nitrogen container and the double-well dish used for warming were placed as close as possible (10–20 cm) to allow for a fast transfer (less than a second), to facilitate a high warming rate. After 1 min, oocytes were carefully moved to a 200-μl droplet of DS, where they remained 3 min. Two washing steps of 5 and 1 min, respectively, were performed in 200 μl droplets of WS. Warmed oocytes remained in culture for 2 h prior to ICSI.

Primary outcomes

Morphological survival

Morphological survival was dictated 2 h post-warming. Oocytes evaluation was based on the integrity of oocyte features, such as intact polar corpuscle, normal oolemma, and absence of vacuoles [32]. Oocytes clearly degenerated or considered not suitable for ICSI were deemed non-viable.

Fertilization rate

Fertilization rate was defined as the proportion of oocytes with two pronuclei at the time of fertilization check (17 ± 1 h post-insemination) [32]. The number of oocytes showing no signal of fertilization, abnormal fertilization (1 or >2 pronuclei), and degenerated oocytes was recorded.

Secondary outcomes

Embryo development was checked at 44 ± 2 h (day 2) and 68 ± 2 h (day 3) post-ICSI. For comparative purposes, the embryo assessment algorithm proposed by Cobo et al. [33] was used, considering day 2 good quality embryos as those presenting 2–4 blastomeres, ≤15% type I–II fragmentation, and no multinucleation. Day 3 good quality embryos were those with 6–8 blastomeres and ≤20% type I–II fragmentation.

Clinical outcomes

Clinical pregnancy was confirmed by ultrasound visualization of a gestational sac with fetal heartbeat. The implantation rate was calculated as the ratio of gestational sacs with fetal heartbeat by the number of embryos transferred. The live birth rate

was calculated as live birth events per embryo transfer, with twin deliveries considered as a single event. Any perinatal complications were recorded.

Statistical analysis

The study was designed to detect a difference of 15% in the primary endpoint, fertilization rate, and between vitrified and fresh donor oocytes ($N = 216$ ICSI'ed), with a power of 80% and a significance level (α) of 0.05. The correlation between qualitative ordinal variables was analyzed using chi-square and Fisher's exact test. All statistical analysis was performed using the IBM SPSS statistics 17.0 package.

Results

The number of subjects included in the study, their age, and the number of oocytes assigned to the recipients is shown on Table 1.

Primary endpoint: fertilization rates

Out of 73 vitrified MII oocytes, 70 (95.9%) presented morphologic survival 2 h post-warming, and 49 of them (70.0%) presented normal fertilization, compared to 105 (71.9%) of 146 MII fresh oocytes ($p > 0.05$) (Table 2).

Secondary endpoints: embryo quality and development

Table 2 reveals no differences ($p > 0.05$) in cleavage rates or the embryo quality of vitrified or fresh oocytes on day 2 or day 3.

Clinical results

Clinical results of the cycles were collected without comparative purposes and are shown on Table 3. They were not subjected to statistical analysis due to the low sample size. Thirty-eight embryos were transferred in total, with a total implantation rate of 44.7% and a clinical pregnancy rate of 52.6%, resulting in 12 live birth events.

Table 1 Characteristics of the donors and recipients included in the study and the ratio of control/experimental oocytes assigned. Oocytes from some donors were assigned to more than one recipient. In the row labelled as "number," the values represent the number of donors or recipients. In the rest of the rows, data is presented as mean (SD)

	Donors	Recipients
Number	13	19
Age (y.o.)	25.6 (2.7)	41.5 (4.2)
# fresh oocytes (control)	—	7.7 (1.2)
# vitrified oocytes (experimental)	—	3.8 (1.1)

Table 2 Oocyte distribution and laboratory outcomes. Results expressed as percentages (%). Morphological survival was dictated 2 h post-warming. Oocytes evaluation was based on the integrity of oocyte features, such as intact polar corpuscle, normal oolemma, and absence of vacuoles. Fertilization rate was defined as the proportion of oocytes with two pronuclei at the time of fertilization check (17 ± 1 h post-insemination). Day 2 good quality embryos were those presenting 2–4 blastomeres, ≤15% type I–II fragmentation, and no multinucleation. Day 3 good quality embryos were those with 6–8 blastomeres and ≤20% type I–II fragmentation

		Fresh	Vitrified	*p* value
# of oocytes		146	73	–
Survival		–	70/73 (95.9)	–
Normal fertilization		105/146 (71.9)	50/70 (71.4)	0.768
Day 2	Cleavage rate	99/105 (94.3)	45/50 (90.0)	0.346
	Good quality embryos	71/99 (71.7)	37/45 (82.2)	0.071
Day 3	Cleavage rate	90/99 (90.9)	39/45 (86.6)	0.451
	Good quality embryos	63/90 (70.0)	27/39 (69.2)	0.171

Discussion

It is well established that human or animal-derived protein supplementation of the solutions used for gamete and embryo vitrification has a positive effect on the survival rates obtained [24]. Solutions are typically supplemented with 10–20% of SSS or DSS—albumin and glycoprotein solutions—that, among other things, provide viscosity to the solution and a surfactant property that enables the handling and pipetting of the gametes/embryos [23]. These formulations have been used for years and are currently in use with very satisfactory results. However, there is an interest in replacing blood-derived components from the vitrification solutions with synthetic substitutes, such as HPC. Desirable advantages that are sought in HPC-supplemented, protein-free solutions are reduced production cost and variability, and eliminating the hypothetical risk of viral contamination associated with protein purified from blood [24]. The addition of hydroxypropyl cellulose instead of serum to vitrification solutions can provide the same viscosity that enables in vitro manipulation and enhances the glass-forming tendency of the solution, without the aforementioned drawbacks [25, 28].

The present study is a direct comparison of the laboratory outcomes of two groups of oocytes: a control group of fresh oocytes and an experimental group of vitrified oocytes using an HPC-supplemented set of vitrification and warming solutions. Oocytes from both groups derived from the same donor were microinjected and cultured in parallel by the same team of embryologists in the same environment, minimizing the

Table 3 Report of the total clinical outcomes of the study patients, and comparison between transfers performed with embryos from the control group of fresh oocytes, embryos from the experimental group of vitrified oocytes, and mixed transfers in which an embryo of each group was replaced

	Total	Fresh	Mixed	Vitrified
Transfers	19	10	8	1
Embryos transferred	38	21	15	2
Embryos transferred (mean per transfer)	2.0	2.1	1.9	2
Clinical pregnancies (%)	10 (52.6)	5 (50.0)	4 (50.0)	1 (100)
Implanted embryos (%)	18 (47.3)	8 (38.1)	8 (53.3)	2 (100)
Live births (%)	8 (42.1)	4 (40.0)	4 (50.0)	1 (100)

number of variables influencing the outcomes of fertilization and embryo development. As mentioned, the two cohorts, even if from the same donor, were recovered from two different stimulation cycles: a source of variability in terms of oocyte quality. Nonetheless, the fact that no differences were observed in between both groups reveals that the process of vitrification and warming did not impair the capacity of the oocytes to fertilize and develop. This outcome is in agreement with the literature [25, 28] and supports the efficacy of HPC-based vitrification solutions.

The primary outcome, the fertilization rate of fresh and vitrified oocytes, was compared without finding any significant differences. The biological process of fertilization, represented by the apparition of the two pronuclei, requires the intervention of complex oocyte machinery that could be damaged during vitrification. A successful fertilization event was used as primary endpoint to assess the survival post-vitrification and warming [12, 13]. A successful fertilization event was used as primary endpoint to assess the survival post-vitrification and warming [12, 13]. In addition, a similar rate of good quality embryo formation on day 2 and 3 occurred between groups. For completeness, a complete follow-up to delivery is also included.

The results obtained in our experiments for survival, fertilization, and cleavage outcomes obtained match the vitrification key performance indicators (KPIs) recently set [32, 34]. Our results are also comparable with previously reported data demonstrating comparable viability and development of vitrified oocytes to fresh controls [33, 35].

Up to now, other authors had presented data showing the effectiveness of protein-free, HPC-supplemented solutions combined with ethylene glycol, dimethyl sulfoxide, and trehalose as the non-permeant cryoprotectant agent. They have compared its results with protein-based solutions: Inoue [26] and Kuwayama [27] presented results from bovine embryos and oocytes and human oocytes, showing comparable survival rates using HPC and SSS supplemented solutions. Mori et al. [25] compared media supplemented at 1 and 5% (*v*/*v*) with a stock solution of 60 mg HPC/ml Milli-Q water against 5–20% SSS supplementation, describing similar physical properties and survival rates in mouse and human oocytes and blastocysts with both solutions. Most recently, a retrospective study by Coello et al. [28] compared the use of solutions supplemented with 0.06 mg HPC/ml and trehalose against 20% SSS + sucrose, showing similar laboratory and clinical outcomes. Our results are the first report of the combination of HPC as a surfactant agent and sucrose as the osmotic agent; as up to now, it only had been tested in combination with trehalose.

Larger prospective randomized studies comparing the outcomes of oocyte vitrification with protein vs. HPC supplementation, in which embryos are cultured to blastocyst stage and the live birth rate is used as a primary endpoint, are the ultimate comparative test and would be necessary. Yet the results reported in this article add to the growing body of evidence documenting efficacy of the use of fully synthetic, protein-free vitrification solutions.

Acknowledgments We would like to give special thanks to all the staff of the clinic that participated in the study. We would also like to express our gratitude for the excellent peer-review process the manuscript was subjected to.

Compliance with ethical standards The study was conducted as described in the protocol, which was developed in accordance to the principles of Helsinki and the national Policy of Good Clinical Practice (ISO 14155:2011), and was approved by the internal review board of the institutional research committee. All recipients were informed thoroughly about the protocol of the study and signed an informed consent form.

References

1. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at −196°C by vitrification. Nature. 1985;313:573–5.
2. Bakhach J. The cryopreservation of composite tissues: principles and recent advancement on cryopreservation of different type of tissues. Organogenesis. 2009;5(3):19–126.
3. Baudot A, Alger L, Boutron P. Glass-forming tendency in the system water-dimethyl sulfoxide. Cryobiology. 2000;40(2):151–8.
4. Benson EE. Cryopreservation theory; plant cryopreservation: a practical guide. Springer 2008
5. Boutron P, Mehl P, Kaufmann A, Angibaud P. Glass-forming tendency and stability of the amorphous state in the aqueous solutions of linear polyalcohols with four carbons. I. Binary systems water-polyalcohol. Cryobiology. 1986;23(5):453–69.

6. Risco R, Elmoazzen H, Doughty M, He X, Toner M. Thermal performance of quartz capillaries for vitrification. Cryobiology. 2007;55(3):222–9.
7. Seki S, Mazur P. Ultra-rapid warming yields high survival of mouse oocytes cooled to −196 °C in dilutions of a standard vitrification solution. PLoS One. 2012;7:e36058.
8. Toner M, Carvalho EG, Karel M. Thermodynamics and kinetics of intracellular ice formation during freezing of biological cells. J App Phys. 1990;67:1582–93.
9. Yavin S, Arav A. Measurement of essential physical properties of vitrification solutions. Theriogenology. 2007;67:81–9.
10. Wowk B. Thermodynamic aspects of vitrification. Cryobiology. 2010;60(1):11–22.
11. Cobo A, Serra V, Garrido N, Olmo I, Pellicer A, Remohí J. Obstetric and perinatal outcome of babies born from vitrified oocytes. Fertil Steril. 2014;102(4):1006–15.
12. 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 favorably with day 5 blastocyst transfer. Hum Reprod. 2002;17:1852–5.
13. Solé M, Santaló J, Boada M, Clua E, Rodríguez I, Martínez 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;28:2087–92.
14. Practice Committee of the American Society for Reproductive Medicine, & Practice Committee of the Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril. 2013;99(1):37–43.
15. Ruffing NA, Steponkus PL, Pitt RE, Parks JE. Osmometric behavior, hydraulic conductivity, and incidence of intracellular ice formation in bovine oocytes at different developmental stages. Cryobiology. 1993;30:562–80.
16. Van den Abbeel E, Schneider U, Liu J, Agca Y, Critser JK, Van Steirteghem A. Osmotic responses and tolerance limits to changes in external osmolalities, and oolemma permeability characteristics, of human in vitro matured MII oocytes. Hum Reprod. 2007;22:1959–72.
17. Fahy GM. The relevance of cryoprotectant "toxicity" to cryobiology. Cryobiology. 1986;23:1–13.
18. Széll A, Shelton JN. Osmotic and cryoprotective effects of glycerolsucrose solutions on day-3 mouse embryos. J Reprod Fertil. 1987;80:309–16.
19. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 2007;67:73–80.
20. Stachecki JJ, Garrisi J, Sabino S, Caetano JP, Wiemer K, Cohen J. A new safe, simple, and successful vitrification method for bovine and human blastocysts. Reprod Biomed Online. 2008;17:360–7.
21. Vanderzwalmen P, Connan D, Grobet L, Wirleitner B, Remy B, Vanderzwalmen S, et al. Lower intracellular concentration of cryoprotectants after vitrification than after slow freezing despite exposure to higher concentration of cryoprotectant solutions. Hum Reprod. 2013;28(8):2101–10.
22. Arav A. Cryopreservation of oocytes and embryos. 40th Anniversary Special Issue. Theriogenology. 2014;81:96–102.
23. Vajta G, Rienzi L, Cobo A, Yovich J. Embryo culture: can we perform better than nature? Reprod Biomed Online. 2010;20(4):453–69.
24. Shaw JM, Kuleshova LL, Macfarlane DR, Trounson AO. Vitrification properties of solutions of ethylene glycol in saline containing PVP, Ficoll, or dextran. Cryobiology. 1997;35:219–29.
25. Mori C, Yabuuchi A, Ezoe K, Murata N, Takayama Y, Okimura T, et al. Hydroxypropyl cellulose as an option for supplementation of cryoprotectant solutions for embryo vitrification in human assisted reproductive technologies. Reprod Biomed Online. 2015;30(6):613–21.
26. Inoue F. Hydroxypropyl cellulose as a macromolecular supplement for cryopreservation by vitrification of bovine ooytes and blastocysts and human oocytes. Fertil Steril. 2011;96(3):S212–3.
27. Kuwayama M. Efficiency of non-protein solutions using hydroxypropyl cellulose on survival of bovine and human oocytes and embryos after vitrification. Fertil Steril. 2013;100(3):S174.
28. Coello A, Campos P, Remohí J, Meseguer M, Cobo A. A combination of hydroxypropyl cellulose and trehalose as supplementation for vitrification of human oocytes: a retrospective cohort study. J Assist Reprod Genet. 2015;33(3):413–21.
29. Clua E, Tur R, Coroleu B, Boada M, Barri PN, Veiga A. Analysis of factors associated with multiple pregnancy in an oocyte donation programme. Reprod Biomed Online. 2010;21:694–9.
30. Risco R, Hebles M, Saa AM, Vilches-Ferron AM, Sanchez-Martin P, Lucena E, et al. Safespeed technology—the myth of ultrahigh cooling rates: a close device and a serum-free media for the vitrification human oocytes/embryos with the highest recovery rates. Hum Reprod. 2013;28(S1):i180.
31. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005;11:300–8.
32. Alpha SIRM. The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting. Reprod Biomed Online. 2012;25(2):146.
33. Cobo A, Kuwayama M, Pérez S, Ruiz A, Pellicer A, Remohí J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 2008;89:1657–64.
34. Maggiulli R, Scarica C, Dovere L, Ievoli E, Ubaldi FM, Rienzi L. Efficiency of human oocyte and embryo vitrification. Curr Trends Clin Embriol. 2015;1:2–11.
35. 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(1):66–73.