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Comparison of two closed carriers for vitrification of human blastocysts in a donor program

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ABSTRACT

The survival of human blastocysts to vitrification with two different carriers is compared. Both vitrification carriers used in this study are in the category of closed carriers, as they completely isolate the samples from direct contact with liquid nitrogen or its vapours during cooling and storage, until warming. This characteristic is appealing because it reduces or eliminates the theoretical risk of cross-contamination during that period of time.

The two closed vitrification systems used present very different design and features: in the High Security Vitrification device, the carrier straw containing the embryos is encapsulated inside an external straw before plunging in liquid nitrogen, resulting in thermal insulation during cooling. On the other hand, in the SafeSpeed carrier embryos are loaded in a thin-walled, narrow capillary designed to maximize the thermal transference. Both closed carriers achieved comparable outcomes in terms of survival of blastocysts to the vitrification process, with 97.5% vs. 96.1% survival with HSV and SafeSpeed, respectively. In conclusion, the cooling and warming rates at which these carriers operate, in combination with the cytosolic solute concentration in the cells of the cryopreserved blastocysts attained after a cryoprotectant-loading protocol, result in successful vitrification of human blastocysts for human assisted reproduction.

1. Introduction

The cryopreservation of human embryos is a key element in the human assisted reproduction field: the ability to preserve the supernumerary embryos that are generated during in vitro fertilization treatments greatly improves treatment options [12]. Cryopreservation is particularly important in donation programs and in cases where the whole cohort of embryos obtained in an in vitro fertilization (IVF) treatment is cryopreserved, in a policy termed 'freeze all' [2,30]. The current method of choice for cryopreservation of human embryos is vitrification, and the clinical embryologist has a wide array of both commercial [8,11,13,15,18,19,36,39] and non-commercial [14,17,23,31,32,34,37] vitrification carriers —the devices in which embryos are loaded prior to cooling, and where they remain stored at cryogenic temperatures until warming— to choose from.

Due to the hypothetical risk of liquid nitrogen mediated cross-contamination during cooling and storage, vitrification carriers have been classified by the level of exposure of the embryos and the media surrounding them to the cooling agent liquid nitrogen [3,16,20,25]. From a lower to higher degree of isolation, there are: fully open carriers, which directly expose the embryo to liquid nitrogen during cooling and storage; open cooling and closed storage carriers; semi-closed, vapor-mediated cooling carriers; closed carriers composed of thin and narrow capillaries; and hermetically-sealed-into-container carriers [38]. There are also alternatives in which liquid nitrogen is sterilized [24].

Whether it is preferable to use open or closed carriers is a hotly debated topic in the human assisted reproduction field [9,10,38,42]. European directives do not impose the use of closed vitrification systems, but recommend laboratories to minimise the risk of contamination of tissues and cells [5]. However, most evidence on the efficiency of vitrification for cryopreservation of oocytes and embryos comes from studies using open-systems [23,26,37]. A recent meta-analysis showed similar survival but a tendency towards lower birth rates in transfers of blastocysts cryopreserved with closed system versus those cryopreserved with open systems, so the equality of both approaches cannot be assumed [41].

However, the performance of a vitrification carrier does not depend on just whether they are open or closed. Aside from the repeatability

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and ease of handling of the carrier to avoid manipulation mistakes, performance will depend on the cooling and, most warming rates it achieves and the volume of the solution containing the embryos that is loaded into it [6,21,27,33,40].

In this study, we compare the outcomes of blastocyst vitrification when using two different closed carriers, with different design: the high security vitrification carrier (HSV, Irvine Scientific, USA), and the capillary-based closed vitrification carrier SafeSpeed (SS, Safepreservation, Spain), specifically designed to maximize heat-transfer efficiency [13].

2. Material and methods

2.1. Study design

This retrospective study reports data from 79 cycles of elective transfers of supernumerary vitrified and warmed blastocyst from a fresh egg donation program. All treatments were performed in Instituto Bernabeu, between January and December 2015, when both devices were used concurrently. The data included in this study was framed in the routine clinical activity and retrospectively collected with Instituto Bernabeu's IRB approval.

2.2. Laboratory procedures

Donor oocytes were fertilized by ICSI, and cultured to the blastocyst stage using microdrops of pre-equilibrated in vitro culture media (CCM, Vitrolife, Sweden), in 5% O_2, 6% CO_2, at 37 °C. Blastocysts were graded according to Istanbul consensus scoring on embryo assessment [1].

Briefly, based on their stage of development blastocyst were graded on a 1–4 scale, with grade 1 equivalent to an early blastocyst with a blastocoel that is half of the volume of the embryo, grade 2 equivalent to a blastocyst with a blastocoel that is greater than half of the volume of the embryo, grade 3 equivalent to an expanded blastocyst, and grade 4 equivalent to a blastocyst hatching or hatched. For each of the developmental stages, the inner cell mass (ICM) and trophectoderm (TE) were graded on a 1–3 scale. ICM grade 1 was defined as prominent, easily discernible, with many compacted and tightly adhered cells; grade 2 as easily discernible, with many loosely grouped cells, and grade 3, in which the ICM was difficult to discern, with few cells. The TE was assessed as follows: grade 1, many cells forming a cohesive epithelium; grade 2, few cells giving it an irregular aspect; or grade 3, very few large cells. Good quality blastocysts, defined as having an ICM and TE grade 1 or 2, were cryopreserved.

2.3. Vitrification and warming

All the blastocysts from each recipient were assigned exclusively to one of the carriers. Both vitrification and warming procedures were carried out according manufacturer's instructions for use. All solutions were at 23–27 °C room temperature except for TS warming solutions, at 37 °C.

2.3.1. Closed carrier 1: SafeSpeed (SS)

Prior to vitrification of the blastocysts with the SS carrier (Fig. 1), the blastocysts were placed in a 200 µl drop of equilibration solution (SS-ES) containing 7.5% v/v ethylene glycol (EG) and 7.5% v/v dimethyl sulfoxide (Me_2SO). The exposure to this solution lasted for a minimum of 8 and a maximum of 14 min, depending on the time the blastocysts took to re-expand to their isosmotic volume in a subjective assessment by the operator. Blastocysts were then transferred to 200 µl of vitrification solution (SS-VS, 15% v/v EG, 15% v/v Me_2SO, 0.5 M sucrose), rinsed repeatedly and loaded by aspiration in the SS capillary. The capillary and the back end of the straw were then thermo-sealed with a specific device [13]. Once hermetically closed, the straw was plunged in liquid nitrogen, keeping the capillary uncovered by the protective cap, and stirred for a few seconds. Then, while submerged in LN_2, the protective cover is slid down before releasing the straw. The total exposure time to the vitrification solution until quenching is of 60–90 s.

For warming, the capillary containing the embryos is exposed, remaining submerged in LN_2, transferred in a fast motion to a nearby sterile water bath at 37 °C, and stirred for two seconds. Afterwards the capillary is cut at the tip above the sealing, and the embryos are expelled in 200 µL of warming solution (SS-TS, 1 M sucrose) at 37 °C. After 1 min, they are rinsed for 3 min in 200 µl dilution solution (SS-DS, 0.5 M sucrose), followed by a 5 min rinse in 200 µl washing solution (SS-WS, no osmotic agents). Vitrification and warming media, and Safespeed carrier device sold by Safepreservation, Spain. All solutions contained 0.06–0.125 mg/mL of hydroxypropyl cellulose (HPC) for viscosity and as surfactant agent.

2.3.2. Closed carrier 2: high security vitrification (HSV)

Blastocysts were placed in a 50 µL drop of equilibration solution (HSV-ES) containing 7.5% (v/v) EG, 7.5% (v/v) Me_sSO in an M-199 HEPES Buffered Medium supplemented with 20% dextran for 7–10 min, until showing signs of re-expansion. They were then transferred to a 50 µL drop of vitrification solution (HSV-VS) containing 15% (v/v) EG, 15% (v/v) Me_sSO, and 0.5 M sucrose, washed thoroughly to eliminate leftover ES, and loaded in the tip of the HSV carrier (Irvine Scientific, USA). This carrier device containing the embryos is inserted in an outer resin straw, which is thermos-sealed before plunging in liquid nitrogen. The procedure, from exposure of the blastocysts to VS until the plunge in liquid nitrogen, is completed in 60–90 s.

For warming, the back end of the outer resin straw is cut open, keeping part of the carrier device containing the embryos submerged in liquid nitrogen at all times. Then, it is removed from the outer straw and the tip submerged in 250 µL of warming solution (HSV-TS; 1 M sucrose), as fast as possible. Blastocysts are recovered from TS in one minute, and transferred to dilution solution (HSV-DS, 0.5 M sucrose, room temperature) for three minutes, followed by 5 min in washing solution (WS, no osmotic agents, room temperature). Vitrification (VitKit - Freeze) and warming (VitKit – Thaw) solutions and HSV carrier sold by Irvine Scientific, USA.

After warming, embryos are then transferred to four well-dishes containing pre-equilibrated culture media (CCM, Vitrolife, Sweden), and cultured at 37 °C, 5% O_2, and 6% CO_2, until the moment of the embryo transfer, when a final survival and quality assessment was performed (> 2 h after warming). Blastocysts were considered as positive for survival and apt for transfer when > 50% of cells survived vitrification.

2.4. Clinical procedures

All donors started stimulation on day 2–4 of menstrual cycle with an initial dose of 150–300 UI/day of FSH (Fostipur®, Angelini-IBSA, Barcelona, Spain) according to antral follicular count (AFC) and body mass index. Donors were monitored from day 5 of stimulation by transvaginal ultrasounds scans every 2 or 3 days and doses were adjusted individually. When lead follicle reached 13–14 mm a GnRH antagonist (Cetrotide®, Merck-Serono, Madrid, Spain) was administered daily, and GnRH agonist (Triptoreline, 0.2 mg, Decapeptyl®, Ipsen Pharma, Barcelona, Spain) was used for final oocyte maturation when at least 3 follicles were > 18 mm in diameter. Oocyte aspiration was performed 36 h after GnRH agonist injection by transvaginal ultrasound-guided needle-aspiration.

Recipients were subjected to substitutive hormonal therapy with either a) transdermal oestradiol (Evopad 50, Janssen-Pharmaceutica, Belgium): applying 50 µg patches from day 1–8 of the cycle, 100 µg from day 9–12, and 150 µg from day 13 onwards. Patches were changed every 48 h; or b) oral oestradiol valerate (Progynova, Delpharm Lille, France): 2 mg daily from day 1–8, 4 mg daily from day 9–12 and 6 mg