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Thermal and clinical performance of a closed device designed for human oocyte vitrification based on the optimization of the warming rate

Miguel Gallardo [a, b], María Hebles [b], Beatriz Migueles [b], Mónica Dorado [b], Laura Aguilera [b], Mercedes González [b], Paloma Piqueras [b], Lorena Montero [b], Pascual Sánchez-Martín [b], Fernando Sánchez-Martín [b], Ramón Risco [a, c, *]

[a] University of Seville, C/ Camino de los Descubrimientos s/n, 41092, Seville, Spain
[b] Ginemed Clínicas, C/ Farmacéutico Murillo Herrera nº 3-5, 41010, Seville, Spain
[c] National Accelerator Centre, C/ Thomas Alva Edison 7, 41092, Seville, Spain

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ABSTRACT

Although it was qualitatively pointed out by Fahy et al. (1984), the key role of the warming rates in non-equillibrium vitrification has only recently been quantitatively established for murine oocytes by Mazur and Seki (2011). In this work we study the performance of a closed vitrification device designed under the new paradigm, for the vitrification of human oocytes.

The vitrification carrier consists of a main straw in which a specifically designed capillary is mounted and where the oocytes are loaded by aspiration. It can be hermetically sealed before immersion in liquid nitrogen for vitrification, and it is warmed in a sterile water bath at 37 °C. Measured warming rates achieved with this design were of 600.000 ºC/min for a standard DMEM solution and 200.000 ºC/min with the vitrification solution for human oocytes.

A cohort of 143 donor MII sibling human oocytes was split into two groups: control (fresh) and vitrified with SafeSpeed device. Similar results were found in both groups: survival (97.1%), fertilization after ICSI (74.7% in control vs. 77.3% in vitrified) and good quality embryos at day three (54.3% in control vs. 58.1% in vitrified) were settled as performance indicators. The pregnancy rate was 3/6 (50%) for the control, 2/3 (66%) for vitrified and 4/5 (80%) for mixed transfers.

1. Introduction

Human oocyte cryopreservation by vitrification is very efficient, and is no longer considered as experimental [23]. As stated by the American Society of Reproductive Medicine guidelines on MII oocyte cryopreservation, there is enough evidence on its safety, with a high similarity between laboratory and clinical outcomes from fresh and vitrified oocytes. However, hitherto most reported data comes from studies using open, plastic-strip vitrification systems [3,25,33]; therefore it is still in doubt whether such efficiency levels can be achieved with a closed vitrification device [6,7,14,19,20,34,35], in which the biological sample does not have direct contact with liquid nitrogen [35].

According to the paradigm of vitrification under which open systems were designed [2,35], the efficacy of a vitrification carrier depends mostly on the cooling rate achieved [1,15]. Therefore, open carriers rely on the direct exposure of the oocytes with liquid nitrogen to maximize such cooling rates [31]. A drawback of this approach is the hypothetical possibility of contamination by infectious microorganisms present in the liquid nitrogen, and the risk of cross-contamination between patients' samples. That said, there is plenty of literature reporting on the efficacy of plastic-strip open systems on both oocytes and embryos and no cases of contamination have been reported [35]. Also, numerous strategies to improve the biosafety of these systems have been developed [9,13,16,21,22], and it is even discussed whether there is a real probability of cross-contamination in the clinical practice [5,35].

To overcome biosafety issues, closed devices have been

* Corresponding author. University of Seville, C/ Camino de los Descubrimientos s/n, 41092, Seville, Spain.
E-mail address: risco@us.es (R. Risco).

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designed to guarantee asepsis, providing isolation to the liquid nitrogen. However this feature compromises their cooling/warming rates and whether they are as efficient as open systems is a subject of debate [14,19,20,35]. Regardless of this, the most recent literature suggests that the vitrification paradigm has changed: studies in murine model have established that the cooling rate is 'of less consequence' for a successful vitrification than that of the warming rate, which now appears to be the dominant variable over the cooling rate [17,30,31], and even over the CPA concentration of the vitrification solution [12,32]. Whether this translates to human oocytes is not fully established, but it is being acknowledged in recent publications [6].

The introduction of this new degree of freedom - the warming rate - makes possible, at least in principle, the specific design of a closed system in which the central idea is not the cooling rate (as is the case in the open devices) but the warming rate. SafeSpeed is a closed vitrification carrier (Safepreservation, Spain) that has been recently designed under the new vitrification paradigm for a maximized warming rate: the biological samples are loaded on a thin, flexible, thermally efficient capillary that yields not only high cooling rates, but most importantly, very high warming rates [27].

In this manuscript a description of the closed carrier for vitrification and the experiments to measure the thermal rates achieved with it are provided. Afterwards, the protocol and the outcomes when used for vitrification of donor oocytes in an IVF programme are presented.

2. Materials and methods

2.1. SafeSpeed closed vitrification carrier

The SafeSpeed is a carrier for the vitrification of biological samples (Fig. 1). The device is composed by a thin capillary, which is assembled into a main straw with a diameter of 24 mm and 135 mm of length. The rear of the straw can be connected to an aspiration system, necessary to load the samples inside the capillary, using two placement marks as reference for its final position (Fig. 1). Once the biological sample is loaded by aspiration the device is sealed at both ends - the capillary and the rear end - to make it hermetically closed. Then, it is ready to be subjected to cooling by plunging the capillary into liquid nitrogen. For subsequent storage, a slidable transparent plastic cover protects the capillary.

For re-warming, the device is quickly transferred from the liquid nitrogen to a 37 °C water bath. Safepreservation, Spain, commercially produces this device, and it holds the CE marking as a product for the cryopreservation of human oocytes and embryos.

2.2. Measurement of thermal performance of the vitrification carrier

i) *Set-up*: For determining the warming rate we used a temperature measuring system consisting of an ultra-fine thermocouple probe (COCO-001; OMEGA Engineering Inc., USA). After the corresponding amplification, the signal was introduced in one of the analogic input channels of a DAC converter (USB.1208LS; Measurement Computing Inc., USA). A program written in Labview 6.0 (reading rate 600 Hz) gives us the whole thermal history of the thermocouple junction.

The thermocouple was introduced inside the SafeSpeed device, and the copper-constantan junction was carefully placed in the position where the oocyte is allocated. Then, the SafeSpeed device was filled with the solution and sealed at the tip of the capillary. Next, the device was quenched in liquid nitrogen, held 10 s (a time that is more than 2 orders of magnitude higher than that necessary to reach the thermal equilibrium with the cryogenic media), and subsequently re-warmed in a 37 °C water bath, exactly as in the standard vitrification/warming protocol for oocytes.

ii) *Warming rates in relation to the time of flight*: To determine the correlation between the time of flight (the motion from the exit of the liquid nitrogen to the entrance into the water bath) and the warming rates achieved, the motion of the capillary was recorded at 480 frames per second (Fig. 2). The ultra-fast video camera, CASIO EX-ZR100, allowed us to visualise, with a temporal resolution of milliseconds, the position of the carrier device, and in particular, the position of the thermocouple junction, placed, as explained above, in the position where the oocyte/embryo would remain. Its optical properties are: 12.1 megapixels, Objective/focal distance (f): 3.0 to 5.9/f = 4.24–53.0 mm and 12.5X optical zoom. The number frames per second (fps) can be chosen between 120, 240, 480 y 1000. In our case we used 480 fps as the best

Fig. 1. SafeSpeed Carrier. Detailed outline of the SafeSpeed carrier and its components. The capillary where the oocytes are introduced is assembled into a main straw with a sealable rear-end and a slide cover for protection of the capillary during storage. 1: Sealable rear-end. 2: Labelling area. 3: Slide protector cover. 4: Ultra-thin capillary. 4.1: Sample placement marks, 4.2: Sealing mark.

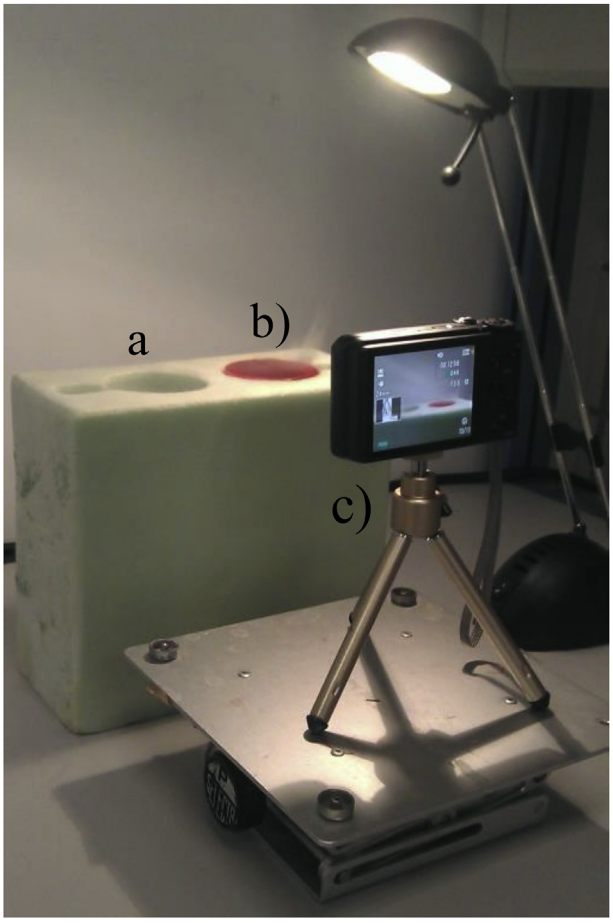

Fig. 2. Set up for image acquisition of the re-warming motion. The following elements are shown: a) Liquid nitrogen container filled to the rim. b) Removable sterilized water bath at 37 °C. c) High speed (1000 fps) video camera (CASIO EX-ZR100). The full motion of the transfer of the capillary containing the thermocouple from the liquid nitrogen to the warm water bath is recorded by the camera.