Cryobiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/cryo

Vitrification of human pronuclear oocytes by direct plunging into cooling agent: Non sterile liquid nitrogen vs. sterile liquid air

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ARTICLE INFO

Keywords:
Human
Oocytes
Embryos
Vitrification
Liquid air
Aseptic

ABSTRACT

In fact, a full sterilization of commercially-produced liquid nitrogen contaminated with different pathogens is not possible. The aim of this study was to compare the viability of human pronuclear oocytes subjected to cooling by direct submerging of open carrier in liquid nitrogen versus submerging in clean liquid air (aseptic system). One- and three-pronuclei stage embryos (n = 444) were cryopreserved by direct plunging into liquid nitrogen (vitrified) in ethylene glycol (15%), dimethylsulphoxide (15%) and 0.2M sucrose. Oocytes were exposed in 20, 33, 50 and 100% vitrification solution for 2, 1 and 1 min, and 30–50 s, respectively at room temperature. Then first part of oocytes (n = 225) were directly plunged into liquid nitrogen, and second part of oocytes (n = 219) into liquid air. Oocytes were thawed rapidly at a speed of 20,000 °C/min and then subsequently were placed into a graded series of sucrose solutions (0.5, 0.25, 0.12 and 0.06M) at 2.5 min intervals and cultured in vitro for 3 days. In both groups, the rate of high-quality embryos (Grade 6A: 6 blastomeres, no fragmentation; Grade 8A: 8 blastomeres, no fragmentation; Grade 8A compacting: 8 blastomeres, beginning of compacting) was noted. The rates of high-quality embryos developed from one-pronuclear oocytes vitrified by cooling in liquid nitrogen and liquid air were: 39.4% ± 0.6 and 38.7% ± 0.8, respectively ($P > 0.1$). These rates for three-pronuclear oocytes were: 45.8 ± 0.8% and 52.0 ± 0.7%, respectively ($P < 0.05$). In conclusion, vitrification by direct submerging of oocytes in clean liquid air (aseptic system) is a good alternative for using of not sterile liquid nitrogen.

1. Introduction

Cryopreservation by direct plunging of human oocytes and embryos into liquid nitrogen as a method of cryopreservation is now the routine in majority of IVF-laboratories [10–16,18,19,21–23,26,29,33,35,39,41].

However, it is not possible to realize a full sterilization of commercially-produced liquid nitrogen contaminated with different pathogens. It is noted by Bielanski [9]: “From a practical point of view, complete sterilization and maintenance of sterility in such a robust system might be a very demanding task, if possible at all. Accordingly, some ubiquitous bacterial agents can be expected in any commercially-produced liquid nitrogen. To this author's knowledge, at the present time, there is no commercial supplier of sterile liquid nitrogen or of a portable device producing liquid nitrogen suitable for assisted reproductive technologies (ART)”.

Recently it was reported about a bench-top device for the production of sterile liquid air [1]. This construction made of two stainless steel containers, one inside the other, having a gap between them that is filled with commercial (not clean) liquid nitrogen. The liquid air is produced inside the cooled, inner stainless steel container, which collects filtered room air (equipped with a 0.22 μm filter) and liquefies it. The liquid air is collected and can be used for cooling of cells by direct contact with this clean cooling agent with the same temperature as liquid nitrogen (−195.7 °C) [1].

The aim of this study was to compare the viability of human pronuclear oocytes subjected to vitrification using cooling by direct

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https://doi.org/10.1016/j.cryobiol.2017.11.009
Received 28 September 2017; Received in revised form 20 November 2017; Accepted 21 November 2017
0011-2240/ © 2017 Published by Elsevier Inc.

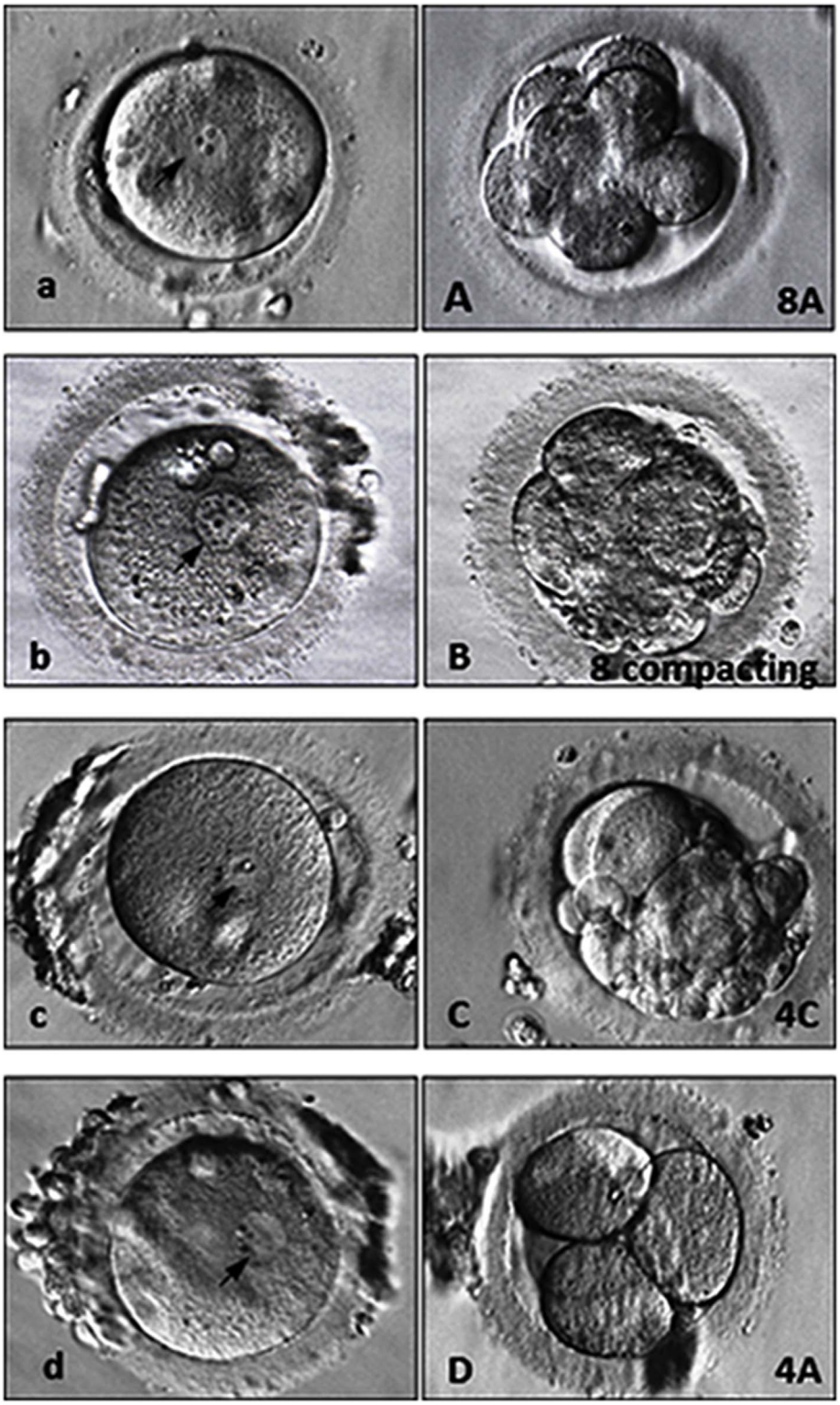

Fig. 1. One-pronuclear oocytes before cryopreservation and 3 Day embryo developed from these oocytes after warming and in vitro culture.
(Arrows) pronucleus.
(a, b, c, d, e) fresh oocytes.
(A, B, C, D, E) 3 day embryo developed from these oocytes, respectively.
(Bottom right) stage and quality of embryos by Steer et al. [36].
Note: Morphologically normal embryos A and D noted as unviable because of developmental arrest.

submerging of open carrier Cryotop™ in liquid nitrogen in comparison with vitrification by submerging of oocytes in clean liquid air (aseptic system).

2. Materials and methods

Except where otherwise stated, all chemicals were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Written informed consent was obtained from the participating couples for the cryopreservation and culture of oocytes. Approvals were obtained from the Ethics Committee of Cologne University, Cologne, Germany and Institute of Biology and Immunology of Reproduction, Sofia, Bulgaria.

2.1. Pronuclear oocytes derivation and embryo evaluation

Patients were stimulated for IVF–ICSI with triptorelin (Decapeptyl w, Ferring, Kiel, Germany) and recombinant follicle-stimulating hormone (Puregonw, Organon, Oss, The Netherlands) according to the

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