Reproductive BioMedicine Online (2014) **28**, 614–623

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ARTICLE

Rapid warming increases survival of slow-frozen sibling oocytes: a step towards a single warming procedure irrespective of the freezing protocol?

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After completing a Master's degree in biology in 1996 at the University of Bologna, Italy, Lodovico Parmegiani completed his postgraduate specialization in biochemistry and clinical chemistry in 2000 at the University of Modena and Reggio Emilia, Italy. In 2008, he received certification as a senior clinical embryologist from the European Society for Human Reproduction and Embryology. In his career he has developed and optimized devices and methods for clinical embryology. His current research interests are cryobiology, gamete selection and micromanipulation. He is currently the Laboratory Director at the Reproductive Medicine Unit, GynePro Medical Centers in Bologna, Italy.

Abstract Nowadays, human oocytes/embryos are cryopreserved via slow freezing or vitrification. The aim of this study was to evaluate a rapid warming protocol for slow-frozen human oocytes based on the standard warming procedure for vitrification. This was a prospective study on 216 sibling oocytes randomized for either conventional rapid thawing or rapid warming with vitrification warming solution. The primary endpoint was morphological assessment of survival at 2 h. Surviving oocytes were divided into two subgroups: (i) parthenogenetically activated; and (ii) fixed and observed for spindle/chromosome configuration. Secondary endpoints were parthenogenetic development and spindle/metaphase configuration. Survival rate with rapid warming was higher (92/102, 90.2%) than with rapid thawing (85/114, 74.6%; $P = 0.005$), and after 3 d of culture the rapidly warmed parthenotes had more blastomeres compared with those rapidly thawed ($P = 0.042$). Meiotic spindle and chromosomal configuration were not significantly influenced by rapid warming or rapid thawing. The finding of this study allows IVF centres to increase the efficiency of oocyte slow freezing, enabling survival rates comparable to vitrification protocols, and potentially to optimize costs by using the same warming protocol for both slow-frozen and vitrified reproductive cells.

KEYWORDS: oocyte slow freezing, oocyte vitrification, parthenogenetic activation, rapid thawing, universal warming protocol, warming

http://dx.doi.org/10.1016/j.rbmo.2014.01.015

Introduction

Nowadays, in assisted reproduction laboratories, human oocytes and embryos are cryopreserved by two main methods: vitrification or slow freezing.

During vitrification, the cells are converted to a glassy state without ice-crystal formation by using a viscous medium with a very high concentration of cryoprotectants (Rall and Fahy, 1985; Yavin and Arav, 2007). However, during slow freezing due to the progressive permeation of some cryoprotectants as the ice crystals form in the cryopreservation solution, a glassy/vitrified state is also obtained within the cell throughout the cooling procedure (Vanderzwalmen et al., 2013). For this reason, since both vitrified and slow-frozen oocytes have a vitrified cytoplasm, it can be postulated that the same warming protocol can be used for both. Furthermore, since for any given concentration of cryoprotectant, the warming rates are much faster than the critical cooling rates (Fahy et al., 1987), the minimal concentration of cryoprotectant to prevent crystallization during warming must be higher than during cooling (Vanderzwalmen et al., 2012). In fact, in most vitrification protocols or commercial kits, the cryoprotectant concentration in the first warming solution is approximately 1 mol/l (The Alpha Consensus Meeting, 2012), which is higher than the cryoprotectant concentration in both the freezing solution and the first thawing solution for rapid thawing of slow-frozen oocytes or embryos (e.g. for oocytes: 0.2—0.3 mol/l in freezing solution, 0.3—0.5 mol/l in thawing solution; Bianchi et al., 2007; Boldt et al., 2006; Fabbri et al., 2001; Parmegiani et al., 2008a). This suggests that vitrification warming solution could be used for thawing slow-frozen oocytes/embryos.

For a number of reasons, but most of all for the guaranteed high survival rate, vitrification is increasingly used worldwide, especially for oocytes (Edgar and Gook, 2012). Nevertheless, to date, perhaps millions of slow-frozen oocytes/embryos have already been stored in IVF cryobanks. Furthermore, IVF centres that have completely switched their cryopreservation programme from slow freezing to vitrification may still receive slow-frozen oocytes/embryos from other centres, cryopreserved by various different protocols. Nowadays, regulations recommend the use of FDA/CE-marked thawing media approved for human IVF; because the shelf life of these media is usually short (some months), it becomes expensive to keep available the appropriate thawing solution for any slow freezing protocol. In this scenario, the possibility of using a 'universal medium' to thaw any cell/tissue irrespective of the freezing protocol may simplify the management of thawing procedures. For all these reasons, the aim of this study is to evaluate a rapid warming protocol for slow-frozen human oocytes based on the standard warming procedure for vitrification, to optimize the survival rate and reduce costs by using the same solutions for both slow freezing and vitrification warming. The rapid warming protocol proposed in this study is potentially applicable also to slow-frozen embryos at any stage of cleavage.

Materials and methods

Study population

Since April 2004, all patients undergoing an IVF treatment at the GynePro Medical Centres, Bologna, Italy have had the option of cryopreserving their surplus oocytes not inseminated during the fresh cycle. This study was performed between December 2012 and January 2013 on 216 sibling oocytes obtained from 40 patients and cryopreserved via slow freezing from April 2004 to November 2008. Patient age (mean ± standard error) at time of freezing was 33.70 ± 0.77 years. The number of oocytes thawed/warmed per patient was 5.40 ± 0.53.

Ethical approval

All the women included in this cryopreservation programme were informed of the procedure and written consent was obtained from each at the time of oocyte freezing. The written consent included the option of oocyte donation for research purposes before destruction, should the patient decide to discontinue the cryostorage. A further agreement was obtained from any patient before starting the study. This study was approved by the Institutional Review Board of the clinic (reference no. 15.10.2012, approved 3 December 2012).

Ovarian stimulation, oocyte retrieval and selection, study design, and endpoints

Ovarian stimulation and oocyte retrieval and selection before cryopreservation were performed as previously described (Parmegiani et al., 2008a). For this study, slow-frozen oocytes were randomized for either conventional rapid thawing or rapid warming via vitrification warming solutions. Randomization was performed by a different embryologist to the operator who performed oocyte thawing/warming using a specific software tool (http://www.randomizer.org): for example, for six straws containing slow-frozen oocytes, a randomized set, 1, 2, 6, was generated, so oocytes in straws 1, 2 and 6 were thawed by conventional rapid thawing and oocytes in straws 3, 4 and 5 were rapidly warmed. The primary outcome measure was morphological assessment of survival at 2 h. Secondary outcome measures were parthenogenetic development and spindle/metaphase configuration.

Cryopreservation

The cryopreservation protocol consisted of a slow-cooling method (Fabbri et al., 2001). Oocyte freezing solutions (OocyteFreeze; Origio, Denmark) contained Dulbecco's phosphate-buffered saline (PBS) supplemented with human serum albumin, alpha- and beta-globulins and 1,2-propanediol (PROH) and sucrose as cryoprotectants.

After washing in a PBS solution (Vial 1—OocyteFreeze; Origio), oocytes were equilibrated for 10 min at room temperature in 1.5 mol/l 1,2-PROH (Vial 2—OocyteFreeze,