



To be presented at the Annual meeting of the Society for Cryobiology, July 20th -23rd, 2008, Charlotte, NC, USA

Improved recovery of human oocytes following non-linear cooling.

G. J. Morris ⁽¹⁾ & M Blayney ⁽²⁾

⁽¹⁾ Asymptote Ltd., St John's Innovation Centre, Cowley Road, Cambridge CB4 0WS, UK

⁽²⁾ Bourn Hall Clinic, Bourn, Cambridge CB3 7TR, UK

Cryopreservation of spermatozoa and embryos is widely used in IVF, by comparison the role of oocyte cryopreservation in assisted reproduction is limited. There are many technical problems associated with cryopreservation of human oocytes either by slow cooling or by vitrification which have limited clinical progress.

Many of the changes in physical properties which occur in an aqueous cryoprotectant following ice nucleation are not linear with temperature. Parameters such as the ice fraction, concentration of solutes include ionic species and cryoprotectants, osmolality, pH, viscosity and gas solubility, all vary in a non-linear manner with temperature. In addition, the biophysical characteristics of cells which determine the response to freezing, for example the cellular permeability to water, also change in a non-linear manner with temperature. Conventional approaches to cryopreservation impose a linear change of temperature with time whilst the stresses that cells are encountering are all non-linear with time. We have demonstrated that improved methods of cryopreservation may be developed for a number of cell-types by specifically manipulating the manner in which cells experience physical changes rather than imposing a linear temperature reduction. In this study we examined human oocytes suspended in a standard cryoprotectant, frozen using a non-linear profile and the effects on post thaw survival and function assessed in comparison with conventional, linear techniques. A new liquid nitrogen free, controlled rate freezer, the Asymptote EF600, was used for both linear and non-linear temperature profiles. The only variable was the temperature profile from -7°C (ice nucleation) to -31°C (initiation of rapid cooling), the elapsed time of the two treatments was identical. The recovery following non linear cooling was significantly improved.

Treatment	Oocyte Recovery	Recovery (%)
Linear cooling	63/101	62%
Non-linear cooling	86/104	83%