Cryobiology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Cryobiology

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

Numerical simulation of cooling rates in vitrification systems used for oocyte cryopreservation ☆

M. Sansinena [a,*], M.V. Santos [b], N. Zaritzky [b], J. Chirife [a]

[a] *Facultad de Ciencias Agrarias, Pontificia Universidad Católica Argentina, Cap. Gral. Ramón Freire 183, 1426 Buenos Aires, Argentina*
[b] *Depto. de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional de La Plata and Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-CONICET), Calle 47 y 116, La Plata 1900, Argentina*

ARTICLE INFO

Article history:
Received 18 November 2010
Accepted 12 April 2011
Available online xxxx

Keywords:
Vitrification
Cooling rates
Oocyte
Cryopreservation
Heat transfer
Finite element method

ABSTRACT

Oocyte cryopreservation is of key importance in the preservation and propagation of germplasm. Interest in oocyte cryopreservation has increased in recent years due to the application of assisted reproductive technologies in farm animals such as in vitro fertilization, nuclear transfer and the need for the establishment of ova/gene banks worldwide. However, the cryopreservation of the female gamete has been met with limited success mainly due to its small surface-area:volume ratio.

In the past decade, several vitrification devices such as open pulled straws (OPS), fine and ultra fine pipette tips, nylon loops and polyethylene films have been introduced in order to manipulate minimal volumes and achieve high cooling rates. However, experimental comparison of cooling rates presents difficulties mainly because of the reduced size of these systems. To circumvent this limitation, a numerical simulation of cooling rates of various vitrification systems immersed in liquid nitrogen was conducted solving the non-stationary heat transfer partial differential equation using finite element method.

Results indicate the nylon loop (Cryoloop®) is the most efficient heat transfer system analyzed, with a predicted cooling rate of 180,000 °C/min for an external heat transfer coefficient $h = 1000$ W/m^2 K when cooling from 20 to −130 °C; in contrast, the open pulled straw method (OPS) showed the lowest performance with a cooling rate of 5521 °C/min considering the same value of external heat transfer coefficient. Predicted cooling rates of Miniflex® and Cryotop® (polyethylene film system) were 6164 and 37,500 °C/min, respectively, for the same heat transfer coefficient.

Introduction

Oocyte cryopreservation is of key importance in the preservation and propagation of the female germplasm in farm animals. Many livestock breeds are experiencing a gradual diminishment of genetic diversity; therefore, it is in the interest of the international community to conserve the livestock genetics.

In order to maintain cell viability, biological functions must be halted by inducing the cell into a suspended animation state by cooling it into a solid phase [13–16]. Compared to the cryopreservation of male gametes, the female gamete represents a greater challenge because the oocyte has a low surface area:volume ratio that makes the cell extremely difficult to dehydrate using traditional, slow cooling cryopreservation protocols [12]. Slow freezing, a common method for cryopreservation of oocytes, has been shown to cause osmotic shock due to solution effects and intracellular ice crystallization leading to cell damage. However, in many species, oocytes are damaged by even after short exposures (less than 1 min) to relatively high temperatures. Chill-sensitive oocytes may however survive cryopreservation if the temperature is very rapidly lowered from a safe temperature (e.g. body temperature) to one which is so low that chemical and biological processes cease. This explains why rapid cooling procedures such as vitrification are proving to be better suited to oocytes than slow cooling.

The preservation of cells and tissues by vitrification was first described by Luyet in 1937 [13,14]. This principle, which involves the solidification of a sample into an amorphous, glassy-state while maintaining the absence of both intracellular and extracellular ice crystals [18], requires high concentrations of cryoprotectants and extremely rapid cooling rates in order to avoid intra and extracellular ice formation.

The reduction of the volume of the vitrification solution results in an increase in cooling and warming rates as well as a reduction in the probability of ice crystal nucleation and formation [7]. It has

---

☆ *Statement of funding:* This study was funded by Pontificia Universidad Católica Argentina, Facultad de Ciencias Agrarias and Centro de Altos Estudios Gándara (CAEG), Buenos Aires, Argentina. Authors have expressed no conflict of interest or financial affiliation with any of the commercial cryopreservation devices compared in the study.

* Corresponding author.
E-mail addresses: msansinena@uca.edu.ar, msansinena@fibertel.com.ar (M. Sansinena).

0011-2240/$ - see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.cryobiol.2011.04.006

been previously stated that, although there is no clear definition of what volume can be defined as "minimum" for vitrification, the most common use of this term refers to devices that handle solution volumes under ≤1 µl solution [8,9,6,10,11,21].

In order to minimize the working volume of the cryoprotectant solution and achieve very high cooling rates, several minimal volume vitrification systems have been commercially introduced in the last years. Although there are different designs of vitrification systems, the basic physical principle behind all of them is to maximize heat transfer during exposure to liquid nitrogen, therefore achieving ultra high cooling rates. Some of these systems include open pulled straws (OPS, generally manufactured 'in laboratory' from polyethylene French straws), nylon loops (Cryoloop™, Hampton Research, CA, USA), polyethylene films (Cryotop®, Kitazato BioPharma Co., Fuji, Japan and McGill Cryoloeaf® (Medicult, Jyllinge, Denmark) and Miniflex® pipette tips (Sorenson Bioscience Inc., UT, USA).

The measurement of actual cooling rates of vitrification devices can only be achieved by a few available systems [21,20] because of their reduced size. This limitation has, for the most part, precluded the comparison of actual cooling rates between different oocyte vitrification systems. In addition, there is a large variability and "technician-dependent" fluctuation in the volume of vitrification solution utilized when loading the oocytes, mainly due to the fact that most systems rely on capillary action. Comparisons between vitrification systems reported in literature show lack of consistency and a wide range in oocyte survival and embryo developmental rates after warming [4].

To date, there have been no randomized, controlled experimental studies comparing different devices [1]. A numerical simulation of cooling rates achieved in vitrification would provide valuable information in order to compare the efficiency of available vitrification systems. Therefore, the objective of this study was to compare the efficiency of various oocyte vitrification systems immersed in liquid nitrogen, (Cryoloop®, Cryotop®, Miniflex® and Open Pulled Straw) by using finite element numerical simulation of their cooling rates obtained from the unsteady-state heat conduction equation for the appropriate geometries of the vitrification systems.

Materials and methods

Description of vitrification devices

(a) The Cryoloop® system (Hampton Research, CA, USA) is a device originally designed for crystallography used to mount, freeze, and secure the crystal during cryocrystallographic procedures and X-ray data collection. It consists of a mounted loop of 20 µm diameter nylon. These nylon loops are pre-staked to hollow, stainless steel MicroTubes™. Oocytes are loaded with a volume <1 µl onto the loop, maintained in the vitrification solution by surface tension and plunged into liquid nitrogen.

(b) The Cryotop® (Kitazato Supply, Inc, JP) or Cryoleaf® (Med-Cult Inc., Jyllinge, Denmark) systems are thin polyethylene film strips of approximately 0.4 mm wide, 20 mm long and 0.1 mm thick described by Kuwayama et al. [6] that allow for the oocytes to be vitrified to be placed in an open drop of minimal volume and then plunged directly into liquid nitrogen.

(c) The Miniflex® polyethylene tips (Sorenson Bioscience, Inc., UT, USA) used for vitrification were reported by Cremades et al. in 2004 [2]. They were described to have 360 µm inner diameter and a 77 µm wall thickness.

(d) The Open Pulled Straw (OPS) are normally manufactured in the laboratory from 0.25-mL polyethylene French straws (I.M.V. Orsay, France), typically by softening over a hot plate and pulling manually until the inner diameter decreases from 1700 to approximately 800 µm and wall thickness of the central part decreases from approximately 150–70 µm. The straws are air-cooled and cut at the narrowest point with a razor blade. Their commercial version, the Cryotip® (Irvine Scientific, CA, USA) are specially designed drawn plastic straws with ultra-fine tips and protective metal cover sleeves.

Physical model system

(a) It was assumed that vitrification avoided ice crystal formation during cooling.
(b) Thermal properties of the working solution were assumed equivalent to water properties since water content of the working solutions is usually very high [19], the model compares the performance of the devices loaded with working solutions and assumes that there is no cell is suspended in the system.
(c) The cooling rate (°C/min) was defined as the time needed to reduce initial core temperature (warmest point of the system) of the liquid from 20 °C to −130 °C while avoiding ice formation (vitrification).
(d) The effect of external heat transfer coefficient, as a main factor affecting results, was also considered in the calculations. In 2008, He et al. [3] developed a thermal model to determine the behavior of quartz micro-capillary systems that are important to achieve ultra-fast cooling; they also predicted the effect of capillary dimensions and thermal properties of the materials used in the devices (i.e. plastic, quartz, sapphire, gold, cooper, silver, diamond, etc.).
(e) Although accurate computations will require functions of the thermal properties of materials involved (water and polyethylene) versus temperature, Sansinena et al. [19] noted that for approximate calculations average values of the thermal properties may be used; mainly considering that the thermal properties of subcooled and vitrified water are yet unknown [3]. Thus, for water the following values corresponding to supercooled water at −23 °C were used: thermal diffusivity (α) 1.205×10^{-7} m^2/s; thermal conductivity (k) 0.50 W/m K, specific heat (C_p) 4218 J/kg K, and density (ρ) 983 kg/m^3. For plastics (polyethylene) the following values at 23–25 °C were used: (α) 1.455×10^{-7} m^2/s; thermal conductivity (k) 0.22 W/m K, specific heat (C_p) 1680 J/kg K, and density (δ) 900 kg/m^3.
(f) Three external heat transfer coefficients (h = 200, 1000 and 2000 W/m^2 K) were considered; they were obtained from values reported by Kida et al. [5] for heat transfer from a horizontal wire plunged in stagnant liquid nitrogen.

Mathematical modelling

The heat conduction equation using constant thermal properties was therefore established as a linear mathematical problem.

Miniflex® and OPS devices: the Miniflex® and OPS devices can be described as two concentric finite cylinders of different materials: the fluid and the straw. The differential equations that represent the heat transfer for both systems in cylindrical coordinates were described [19] considering radial and axial coordinates. The total volume charge (vitrification solution and oocytes) in the Miniflex® was 0.5 µl, and the calculated L/D (longitude:diameter ratio) ratio was 13. This value is sufficiently large to assume that the axial heat flow contribution is negligible. As a result the system can be numerically solved as a one-dimensional heat conduction problem in an infinite cylinder (see Eqs. (1) and (2).

Please cite this article in press as: M. Sansinena et al., Numerical simulation of cooling rates in vitrification systems used for oocyte cryopreservation, Cryobiology (2011), doi:10.1016/j.cryobiol.2011.04.006

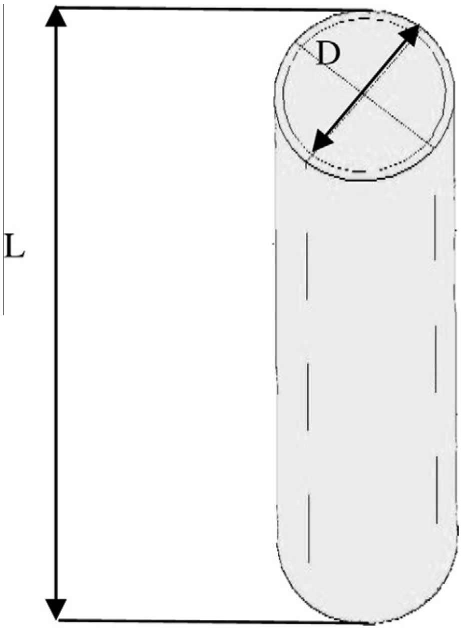


Fig. 1a. Finite cylinder geometry used in Miniflex®.

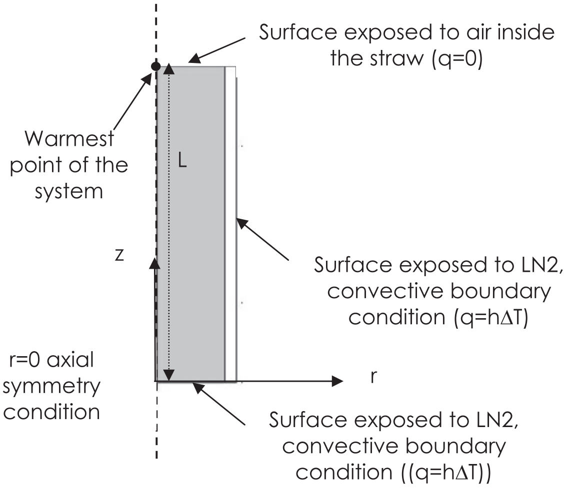


Fig. 1b. OPS geometry and boundary conditions considered to establish the mathematical model.

$$\rho_f\, Cp_f \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_f\, r \frac{\partial T}{\partial r}\right) \tag{1}$$

$$\rho_p\, Cp_p \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_p\, r \frac{\partial T}{\partial r}\right) \tag{2}$$

where ρ corresponds to the density, C_p specific heat, T is temperature and k thermal conductivity and the subscripts f and p to the fluid or plastic material. The temperature change inside the Miniflex® is therefore a function of the radius (r), which is the independent variable (Fig. 1a).

With respect to OPS method, two volumes (1 and 2 µl) were assumed and the L/D ratios were calculated; the obtained values were L/D (1 µl) = 2486 and L/D (2 µl) = 4973. These values of L/D determine the system cannot be approximated as infinite cylinders. The cryogenic solution is loaded by capillarity into the plastic straw reaching a certain height, for a volume of 1 µl the height calculated was $L = 0.002$ m, considering the geometrical parameters of the OPS method (internal and external radius of 400 and 470 µm, respectively). Since a finite cylinder must be assumed, the surface exposed to liquid nitrogen is the bottom circle and the lateral plastic cylinder, the top circle was considered isolated ($q = 0$) since the liquid is in contact with air inside the straw (Fig. 1b).

Numerical simulations were carried out considering both systems, finite cylinder for OPS and infinite cylinder for the Miniflex® device. The equations that represent the finite cylinder were explained in detail in Lane et al. [10].

$$\rho_f\, Cp_f \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_f\, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_f\, r \frac{\partial T}{\partial z}\right) \quad \text{in } \Omega\, f \quad t > 0 \tag{3}$$

$$\rho_p\, Cp_p \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_p\, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_p\, r \frac{\partial T}{\partial z}\right) \quad \text{in } \Omega\, p \quad t > 0 \tag{4}$$

where subscripts f and p correspond to the fluid or to the plastic material domains.

Cryoloop®: the Cryoloop® (Fig. 2a) which has a minimum thickness (e) with respect to the diameter of the system ($e < 20$ µm), can be approximated as a one dimensional heat flow system in Cartesian coordinates (Fig. 2b). The partial differential equation that represents the heat conduction in transient state is: Q1

$$\rho Cp \frac{\partial T}{\partial t} = k \frac{\partial^2 T}{\partial x^2} \tag{5}$$

Cryotop®: in order to obtain accurate numerical predictions the shape of the cryogenic solution (drop) placed on top of the plastic support was considered. This system can be described as an irregular bi-dimensional axial-symmetric problem which can be numerically solved as explained in Lane et al. [10]. The following figure shows the geometry of the system used to simulate the heat transfer in the device as well as the temperature distribution in both domains, the solution and the plastic support. In Fig. 3b) the warmest point in the drop (indicated by an arrow) was used to calculate the minimum cooling times.

For all the devices studied the differential equations that represent the system were numerically solved using the finite element method in COMSOL Multiphysics 3.4. The domain was discretized

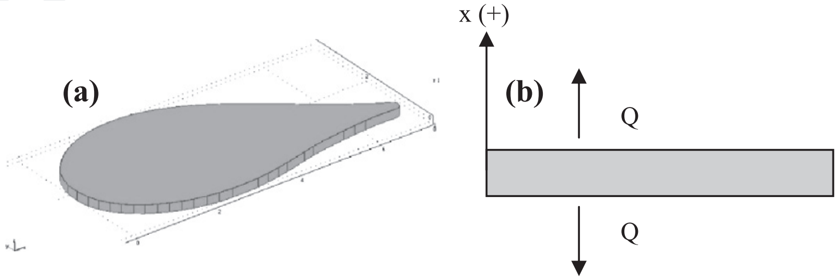
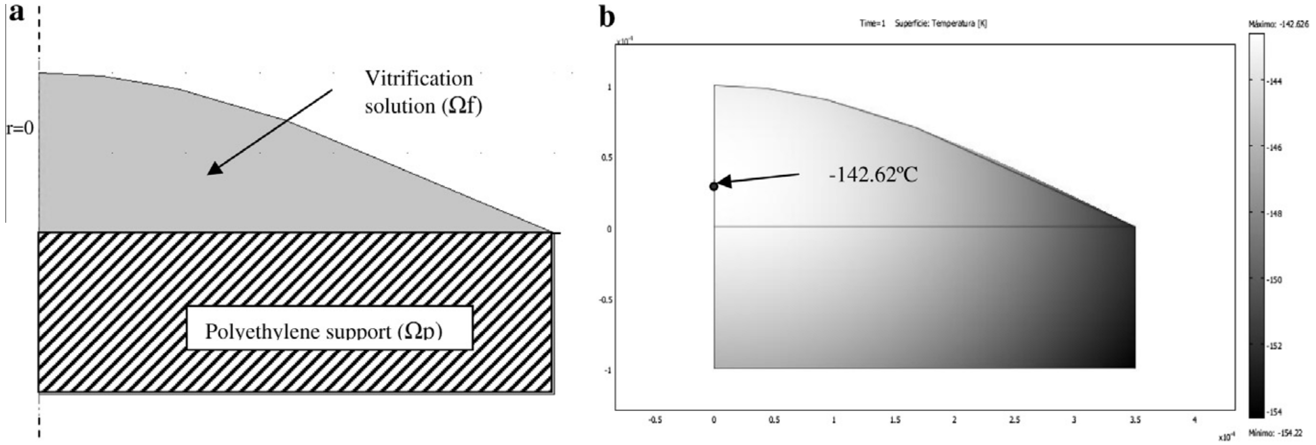


Fig. 2. (a) Cryloop® geometry (b) approximation of the Cryoloop® to 1D geometry (infinite slab).

Please cite this article in press as: M. Sansinena et al., Numerical simulation of cooling rates in vitrification systems used for oocyte cryopreservation, Cryobiology (2011), doi:10.1016/j.cryobiol.2011.04.006



Q4 **Fig. 3.** (a) Cryotop® device, 2D axial symmetric geometry used in the numerical simulation; (b) Temperature distribution after 1 s in the cryogenic solution considering *h* = 200 W/m^2 K.

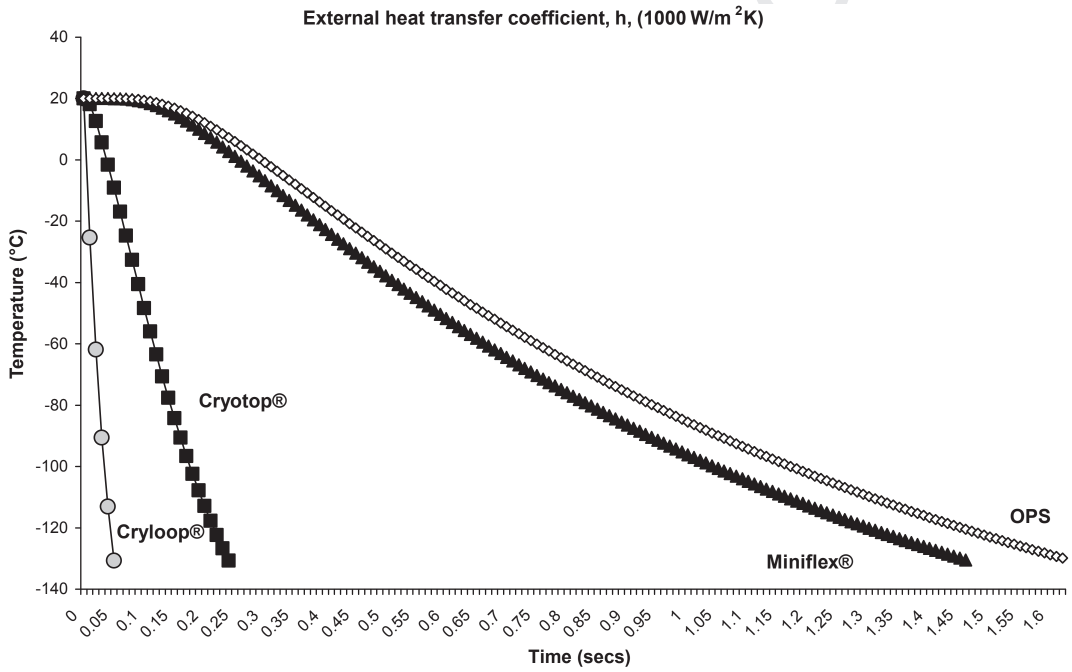


Fig. 4. Numerical simulation of the rate of temperature decrease at the warmest point of different vitrification systems immersed in liquid nitrogen, for an external heat transfer coefficient (*h* = 1000 W/m^2 K).

in triangular (OPS, and Cryotop®) and linear elements (Miniflex®, Cryoloop®) in order to obtain accurate numerical approximations. En each case the warmest point of the system was identified to determine the time–temperature curve that allows the evaluation of the slowest cooling rate (worst condition).

Results

The predicted rates of temperature change (between 20 and −130 °C) in the different vitrification systems immersed in liquid nitrogen are compared in Fig. 4. For a constant external heat transfer coefficient, *h* = 1000 W/m^2 K, the highest rate of temperature change was observed for the Cryoloop® system, while the lowest one corresponded to the OPS.

The cooling rates (°C/min), defined as the time needed to reduce initial temperature at the warmest point of the system from 20 to −130 °C for the different vitrification devices, and considering three values of the external heat transfer coefficient are compared in Table 1. For all the tested heat transfer coefficients the Cryoloop® is the most efficient cooling device system, with a predicted cooling rate of 180,000 °C/min for *h* = 1000 W/m^2 K. In contrast, the OPS system shows the lowest performance, with a cooling rate

Table 1
Predicted cooling rates of the different vitrification systems immersed in liquid nitrogen at various external heat transfer coefficients.

	External heat transfer coefficient, *h* (W/m^2 K)		
	200	1000	2000
Cooling rate, °C/min (from 20 to −130 °C)			
OPS (1 μl)	1694	5521	7826
Miniflex®	1848	6164	8738
Cryotop® (100 μm)	10,465	37,500	60,000
Cryoloop®	36,000	180,000	180,000

Please cite this article in press as: M. Sansinena et al., Numerical simulation of cooling rates in vitrification systems used for oocyte cryopreservation, Cryobiology (2011), doi:10.1016/j.cryobiol.2011.04.006

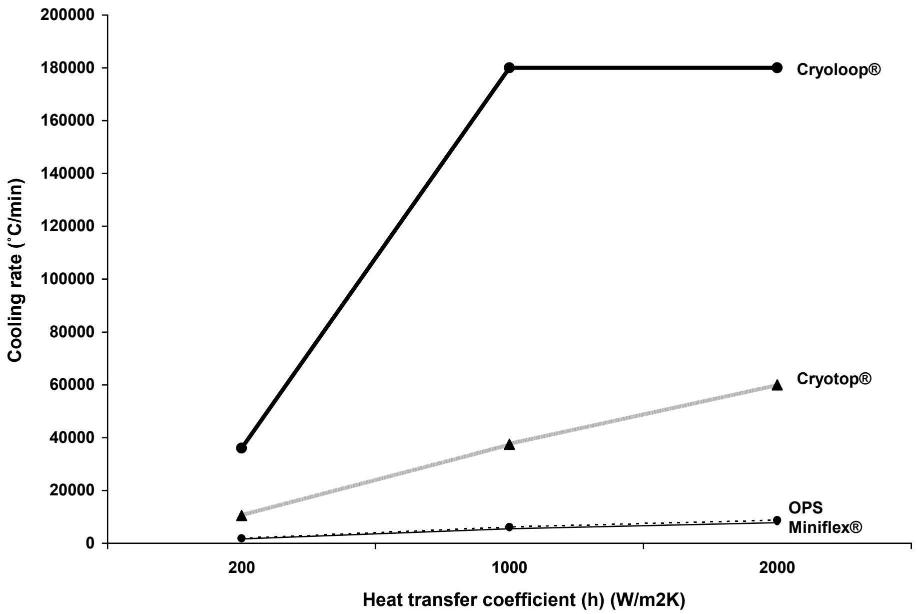


Fig. 5. Effect of external heat transfer coefficient (*h*) on cooling rates of Cryloop®, Miniflex®, Cryotop®, and Open Pulled Straw (OPS).

of only 5521 °C/min for the same external heat transfer coefficient. Rates of temperature change for the Miniflex® are comparable with the OPS; however the Cryotop® (100 μm) cooling rates are at least 6 times faster than OPS for all *h* values considered (Table 1 and Fig. 4).

The core temperature (warmest point of the system) represented the geometrical center with the exception of the Cryotop® and OPS. One of the interesting findings of solving the mathematical model applied to the Cryotop® (irregular bi-dimensional axial symmetric problem) shows that the warmest point shifts during the process (Fig. 3b). On the other hand, the warmest point in the OPS method is positioned at $r = 0$ and $z = L$ (Fig. 1b) for all *h* values and conditions considered in the present work.

The effect of the external heat transfer coefficient (ranging between 200 to 2000 W/m^2 K) on cooling rates of the different vitrification devices (Miniflex®, Cryotop®, Cryoloop® and OPS) when immersed in liquid nitrogen is shown in Fig. 5. For all of the compared systems, increasing the external heat transfer coefficient increases cooling rates. Interestingly, the effect was more pronounced for the Cryotop® than for the OPS and Miniflex® systems, suggesting that the loaded volume into the vitrification device could be one of the main controlling steps.

Discussion

In 2008, He et al. [3] reported a thermal model to determine the behavior of quartz micro-capillary systems; they also predicted the effect of capillary dimensions and thermal properties of the materials used in the devices (i.e. plastic, quartz, sapphire, gold, cooper, silver, diamond, etc.). Certainly, and as noted by He et al. [3] when developing the heat transfer model for cooling quartz microcapillaries, there is some uncertainty in the values of the external heat transfer coefficients in liquid nitrogen. The *h* values adopted in the present work for the different vitrification devices (200–2000 W/m^2 K) correspond to heat transfer coefficients for a horizontal thin cylindrical wire plunged in static liquid nitrogen [5]. On the contrary, He et al. [3] plunged their capillary systems in liquid nitrogen at a high speed to create a convective flow of liquid nitrogen, and assumed an average convective heat transfer, $h = 10{,}000$ W/m^2 K for their thermal analysis.

The performance of Cryoloop® can be attributed to the absence of a solid support and the extremely small dimensions of the vitrification system; its predicted cooling rate (i.e. 180,000 °C/min) is above the value of 100,000 °C/min which may be considered as ultra-fast cooling [3]. Kuwayama [6] suggested that cooling rates of the Cryotop® method may produce cooling rates as high as 40,000 °C/min, and Vajta et al. [21] and Vajta and Kuwayama [20] indicated that OPS method may render high cooling rates over 20,000 °C/min, however this value is higher than the result predicted in our work and shown in Table 1, which corresponds to the cooling rates at the warmest point in the vitrification systems. However if a point in direct contact with LN$_2$ is considered in the OPS system, for example $z = 0$ and $r = 0$ (see Fig. 1b), the cooling rates are 2244, 10,344 and 19,148 °C/min for h values of 200, 1000, and 2000, respectively. These cooling rates would therefore be in agreement with the values reported in literature. With the exception of Cryloop®, for which cooling rate reaches a constant value at intermediate ($h = 1000$ W/m^2 K) and high ($h = 2000$ W/m^2 K) heat transfer coefficients, in all the other vitrification systems the cooling rates increased with higher external heat transfer coefficients.

Conclusions

The performance of different oocyte vitrification systems immersed in liquid nitrogen, (Cryoloop® Cryotop® Miniflex® and Open Pulled Straw) were compared by using numerical simulation of their cooling rates. Thermal histories were obtained from the numerical solution of the unsteady-state heat conduction partial differential equation for the appropriate geometries of the analyzed vitrification devices, using the finite element method.

Results showed that at a constant heat transfer coefficient the highest cooling rate (180,000 °C/min for $h = 1000$ W/m^2 K) was observed for the Cryoloop® system and the lowest rate (5521 °C/min, for the same h value) corresponded to the OPS. The Cryotop® exhibited the second best cooling rate of 37,500 °C/min, whereas the Miniflex® only achieved a cooling rate of 6164 °C/min. It can be concluded that in cryopreservation systems, in which experimental comparison of cooling rates show difficulties mainly because of the reduced size of the vitrification devices, the

Please cite this article in press as: M. Sansinena et al., Numerical simulation of cooling rates in vitrification systems used for oocyte cryopreservation, Cryobiology (2011), doi:10.1016/j.cryobiol.2011.04.006

numerical simulations and the analysis of the predicted thermal histories could contribute to determine the performance of the different techniques.

Uncited reference

Q2 [17].

References

[1] R.C. Chian, P. Quinn, in: R.C. Chian, P. Quinn (Eds.), Fertility Cryopreservation, Q3 Cambridge University Press, pp. 134.
[2] N. Cremades, M. Sousa, J. Silva, P. Viana, S. Sousa, C. Olivera, J. Texeira da Silva, A. Barros, Experimental vitrification of human compacted morulae and early blastocysts using small diameter plastic micropipettes, Hum. Reprod. 19 (2004) 300–305.
[3] X. He, E.Y.H. Park, A. Fowler, L.M. Yarmush, M. Toner, Vitrification by ultra-fast cooling at a low concentration of cryoprotectants in a quartz microcapillary: a study using murine embryonic stem cells, Cryobiology 56 (2008) 223–232.
[4] J.K. Jain, R.P. Paulson, Oocyte cryopreservation, Fert. Ster. 86 (2006) 1037–1046.
[5] M. Kida, Y. Kikuchi, O. Takahashi, I. Michiyoshi, Pool-boiling heat transfer in liquid nitrogen, J. Nucl. Sci. Tech. 18 (1981) 501–513.
[6] M. Kuwayama, Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method, Theriogenology 67 (2007) 73–80.
[7] M. Kuwayama, P. Holm, H. Jacobsen, T. Greve, H. Callesen, Successful cryopreservation of porcine embryos by vitrification, Vet. Rec. 141 (1997) 365.
[8] M. Kuwayama, G. Vajta, O. Kato, S.P. Leibo, Highly efficient vitrification method for cryopreservation of human oocytes, Reprod. Biomed. Online 11 (2005) 300–308.
[9] M. Kuwayama, G. Vajta, S. Ieda, O. Kato, Comparison of open and close methods for vitrification of human embryos and the elimination of potencial contamination, Reprod. Biomed. Online 11 (2005) 608–614.
[10] M. Lane, B. Bavister, E. Lyons, K. Forest, Container-less vitrification of mammalian oocytes and embryos, Nat. Biotech. 17 (1999) 1234–1236.
[11] M. Lane, W. Schoolcraft, D. Gardner, Vitrification of mouse and human blastocyst using a novel cryoloop container-less technique, Fertil. Steril. 72 (1999) 1073–1078.
[12] S.P. Leibo, A. Martino, S. Kobayashi, J.W. Pollard, Stage-dependent sensitivity of oocytes and embryos to low temperatures, Anim. Reprod. Sci. 42 (1996) 45–53.
[13] B.J. Luyet, The vitrification of organic colloids and of protoplasm, Biodynamica 1 (1937) 1–14.
[14] B.J. Luyet, A. Hoddap, Revival of frog's spermatozoa vitrified in liquid air, Proc. Meet. Soc. Exp. Biol. 39 (1938) 433–434.
[15] P. Mazur, Cryobiology: the freezing point of biological systems, Science 168 (1970) 939–949.
[16] P. Mazur, Limits to life at low temperatures and at reduced water contents and water activities, Orig. Life 10 (1980) 137–159.
[17] W.F. Rall, Factors affecting the survival of mouse embryos cryopreserved by vitrification, Cryobiology 24 (1987) 387–402.
[18] W.F. Rall, G.M. Fahy, Ice-free cryopreservation of mouse embryos at −196 °C by vitrification, Nature 313 (1985) 573–575.
[19] M. Sansinena, M.V. Santos, N. Zaritzky, R. Baeza, J. Chirife, Theoretical prediction of the effect of heat transfer parameters on cooling rates of liquid filled plastic straws used for cryopreservation of spermatozoa, Cryo Letters 31 (2010) 120–129.
[20] G. Vajta, M. Kuwayama, Improving cryopreservation systems, Theriogenology 65 (2006) 236–244.
[21] G. Vajta, P. Holm, M. Kuwayama, P.J. Booth, H. Jacobsen, T. Greve, Open pulled straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos, Mol. Reprod. Dev. 51 (1998) 53–58.

Please cite this article in press as: M. Sansinena et al., Numerical simulation of cooling rates in vitrification systems used for oocyte cryopreservation, Cryobiology (2011), doi:10.1016/j.cryobiol.2011.04.006