Asociación Argentina
de Mecánica Computacional

Mecánica Computacional Vol XXXI, págs. 3873-3882 (artículo completo)
Alberto Cardona, Paul H. Kohan, Ricardo D. Quinteros, Mario A. Storti (Eds.)
Salta, Argentina, 13-16 Noviembre 2012

MATHEMATICAL MODELING OF THE HEAT TRANFER PROCESS IN VITRIFICATION DEVICES USED FOR OOCYTE CRYOPRESERVATION

María V. Santos[a], Marina Sansinena[b] , Jorge Chirife[b] and Noemí Zaritzky[a]

[a]*Dto. 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* http://www.cidca.org.ar

[b]*Facultad de Ciencias Agrarias, Pontificia Universidad Católica Argentina, Cap. Gral. Ramón Freire 183, (1426), Buenos Aires, Argentina.* http://www.uca.edu.ar

Keywords: Oocyte cryopreservation, numerical simulation, finite element method, heat transfer.

Abstract. Oocyte cryopreservation is of key importance in the preservation and propagation of germplasm, however, the cryopreservation of the female gamete has been met with limited success. 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 overcome this limitation, the cooling rates of various vitrification systems immersed in liquid nitrogen, (Cryoloop®, Cryotop® ,Miniflex® and Open Pulled Straw) were mathematically modeled using the finite element method by numerically solving the unsteady-state heat conduction partial differential equations for the appropriate geometries of the devices. The thermo-physical properties, thermal conductivity, density and specific heat, which are involved in the partial differential equations, were considered constant since during vitrification there is no phase change of water into ice crystals. The Cryoloop® system (Hampton Research, USA) consists of a mounted nylon loop where the oocytes are loaded and plunged into liquid nitrogen. The Cryotop® (Kitazato Supply, Inc, JP) is a thin polyethylene film strip that holds an open drop with oocytes in a minimal volume before being plunged directly into liquid nitrogen. The Miniflex® polyethylene tips (Sorenson Bioscience, Inc., USA) used for vitrification have 360 µm inner diameter and 77 µm wall thickness. The Open Pulled Straw (OPS) is normally manufactured in the laboratory from 0.25 mL polyethylene French straws (I. M. V., Orsay, France). Results showed that at a constant heat transfer coefficient ($h=1000$ W/m^{2}K)the highest cooling rate (180000 °C/min) was observed for the Cryoloop® system and the lowest rate (5521°C/min) corresponded to the OPS. The Cryotop® exhibited the second best cooling rate of 37500 °C/min, whereas the Miniflex® only achieved a cooling rate of 6164 °C/min. It can be concluded that in cryopreservation systems, the information obtained using the numerical model enables biotechnologist in reproductive areas to determine the performance of each oocyte vitrification device under different operating conditions.

1 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 (Luyet, 1937; Luyet and Hoddap, 1938; Mazur, 1970; Mazur, 1980).

The preservation of cells and tissues by vitrification was first described by Luyet (1937). 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 (Rall and Fahy, 1985), requires high concentrations of cryoprotectants and extremely rapid cooling rates in order to avoid intra and extra-cellular 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 (Kuwayama et al., 1997). 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 (Vajta et al., 1998; Vajta and Kuwayama, 2006) 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 (Jain and Paulson, 2006).

To date, there have been no randomized, controlled experimental studies comparing different devices (Chian and Quinn, 2010). 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 solution of the unsteady-state heat conduction equation for the appropriate geometries of the vitrification systems.

2 MATERIALS AND METHODS

2.1 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® (MedCult 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 (2007) 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. (2004). 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 to 70 µm. The straws are air-cooled and cut at the narrowest point with a razor blade. Their commercial version, the Cryotip® (Irvine Scentific, CA, USA) are specially designed drawn plastic straws with ultra-fine tips and protective metal cover sleeves.

2.2 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 (Sansinena et al., 2010), the model compares the performance of the devices loaded with working solutions and assumes that there is no cell suspended in the system;

c) The cooling rate (ºC/min) was defined as the time needed to reduce initial core temperature (warmest point of the system) of the liquid from 20 ºC to -130 ºC 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. He et al. (2008) 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. (2010) 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 (He et al., 2008). Thus, for water the following values corresponding to supercooled water at -23 ºC were used: thermal conductivity (k) 0.50 W/m K, specific heat (Cp) 4218 J/ kg K, and density (ρ) 983 kg/m^3. For plastics (polyethylene) the following values were used: thermal conductivity (k) 0.22 W/m K, specific heat (Cp) 1680 J/ kg K, and density (ρ) 900 kg/m^3;

e) 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. (1981) for heat transfer from a horizontal wire plunged in stagnant liquid nitrogen.

3 MATHEMATICAL MODELING AND TEMPERATURE SIMULATIONS

The heat conduction equation using constant thermal properties was therefore established as the linear mathematical problem to be solved. 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 using Lagrange elements of order 2, applying a triangular mesh for the OPS, Cryotop® and Miniflex® devices, and one dimensional mesh for the Cryoloop® device. 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).

3.1 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 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 using the following equations which represent the energy transfer in an infinite cylinder without axial energy contribution (see Eq. 1 and 2).

$$\rho_f \; Cp_f \frac{\partial T}{\partial t} = k_f \; \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} \tag{1}$$

$$\rho_p \; Cp_p \frac{\partial T}{\partial t} = k_p \; \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} \tag{2}$$

where ρ is the density, Cp the specific heat, T is temperature and k thermal conductivity; the subscripts f and p correspond to the fluid or the plastic material respectively. The temperature change inside the Miniflex® is therefore a function of “x” and “y”, which are the independent variables. Figure 1 shows the geometry used in the numerical simulations and the temperature distribution after 5s of being submerged in liquid N_2 at -196ºC considering h=200 W/m^2 K. A triangular mesh using Lagrange elements of order 2 was applied to discretize the domains.

With respect to OPS, two volumes (1 and 2 µl) were assumed and the L/D ratios were calculated; the obtained values were L/D (1 µl)=2.486 and L/D (2 µl)=4.973. These values of L/D determine that 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, therefore the heat flux (q) adopted a fixed cero value (q=0) at this interface since the liquid is in contact with air inside the straw (Figure 2).

Numerical simulations were carried out considering both systems, finite cylinder for OPS (Figure 3) and infinite cylinder for the Miniflex® device. The finite cylinder geometry can be considered a solid of revolution (bi-dimensional axial-symmetric problem), therefore the equations that represent these systems using cylindrical coordinates are:

$$\rho_f \, Cp_f \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r} \; k_f \, r \frac{\partial T}{\partial r} \; + \frac{\partial}{\partial z} \; k_f \, r \frac{\partial T}{\partial z} \tag{3}$$

$$\rho_p \, Cp_p \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r} \; k_p \, r \frac{\partial T}{\partial r} \; + \frac{\partial}{\partial z} \; k_p \, r \frac{\partial T}{\partial z} \tag{4}$$

where subscripts f and p correspond to the fluid or to the plastic material domains. A triangular mesh using Lagrange elements of order 2 was applied.

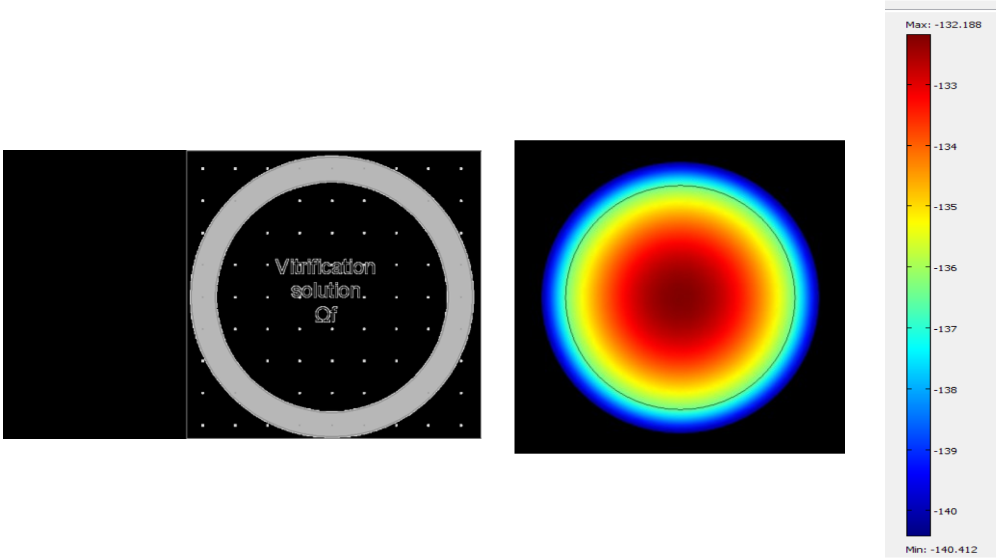

Figure 1. Infinite cylinder geometry used in Miniflex® and Temperature distribution in the Miniflex ® device considering h=200 W/m2 K after 5s of being submerged in liquid N2 at -196° C.

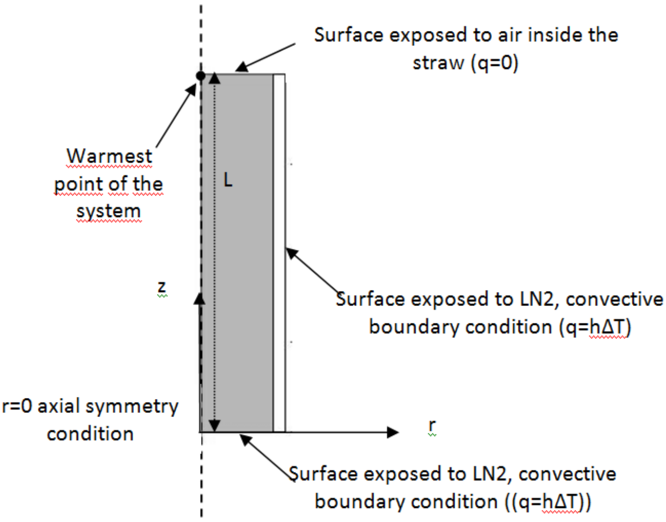

Figure 2. OPS geometry (finite cylinder) and boundary conditions considered to establish the mathematical model.

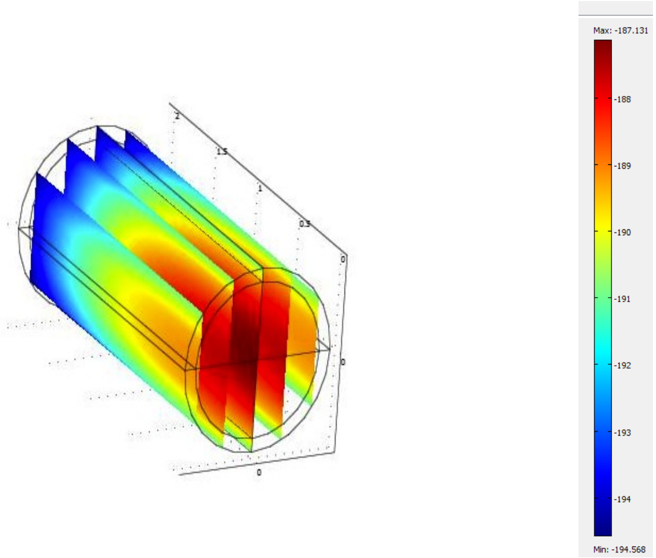

Figure 3. Temperature distribution in the OPS device considering h=1000 W/m2 K after 4s of being submerged in liquid N2 at -196° C

3.2 Cryoloop®:

The Cryoloop® (Figure 4 a) 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 (Figure 4 b) using quadratic 1D elements to discretize the domain. The partial differential equation that represents the heat conduction in transient state is:

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

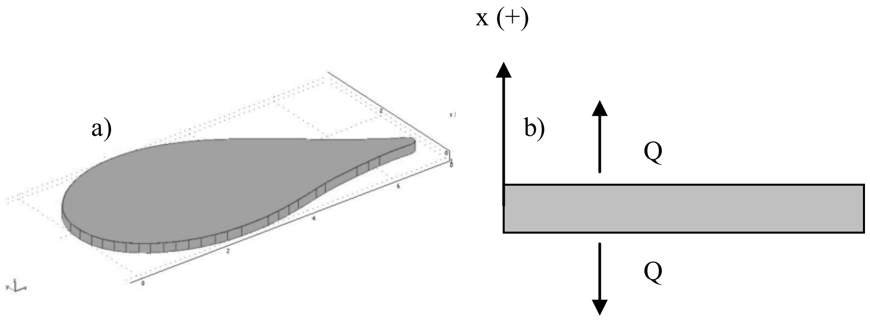

Figure 4. a) Cryloop® geometry b) approximation of the Cryoloop® to 1D geometry (infinite slab)

3.3 Cryotop®:

In order to obtain accurate numerical predictions the shape of the cryogenic solution (drop) placed on top of the plastic support was considered. Two different drop volumes were considered, 0.0171 µl and 0.00516 µl, which correspond to a drop height of 100 and 50 µm respectively.

This system can be described as an irregular bi-dimensional axial-symmetric problem; the equations that constitute the mathematical description are therefore the same as in the OPS device. Figure 5 shows the geometry of the system considering a height of 100 µm (Volume=0.0171 µl) that was 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 Figure 5 the warmest point in the drop (indicated by a dot=P_{hot}) was selected to calculate the minimum cooling times and it was located at r=0 $z=3.2\times10^{-5}$m. Additionally, the temperature distribution was calculated (Figure 6) for two different drop volumes considering h=200 W/m^2 K after 1s of being submerged in liquid N_2.

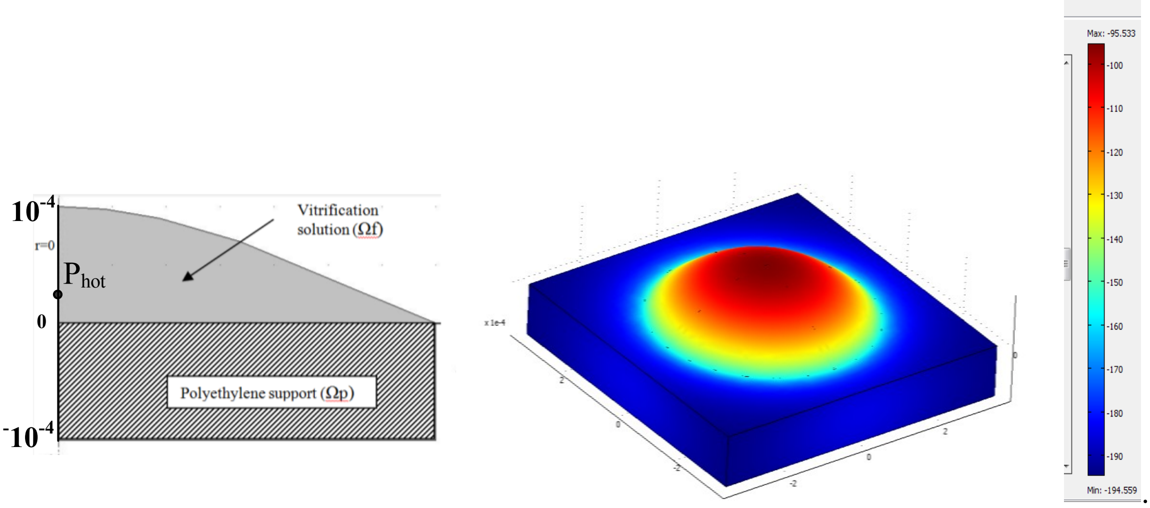

Figure 5. Cryotop® device, 2D axial symmetric geometry used in the numerical simulation and Temperature distribution after 1s in the cryogenic solution considering h=200 W/m^2 K.

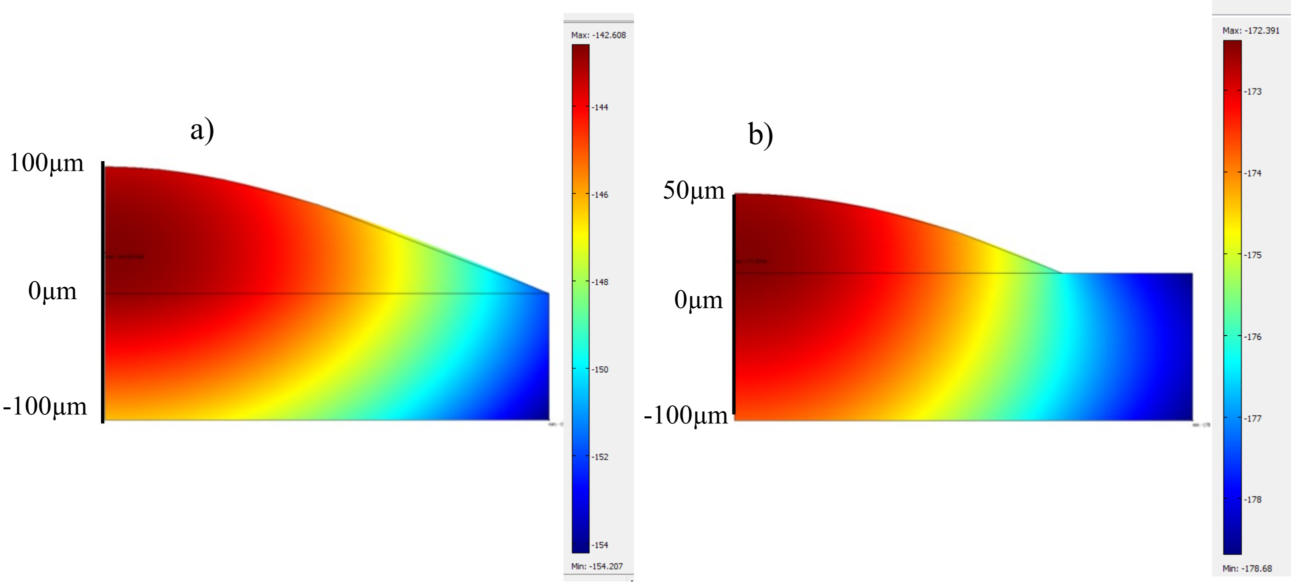

Figure 6. Cryotop® device, Temperature distribution after 1s in the cryogenic solution considering h=200 W/m^2 K for two drop volumes a) 0.0171 µl, drop height 100 µm and b) 0.00516 µl, drop height 50 µm

4 COOLING RATES

In the present work, the average linear cooling rates (ºC/min) at the warmest point of each system (core temperature) were expressed as the quotient between the change in temperature (20 ºC to -130 ºC= 150 ºC) and the elapsed time. Predicted values in the different vitrification systems immersed in liquid nitrogen are compared in Figure 7 for different external heat transfer coefficients (h = 200, 1000 and 2000 W/m^2 K). The highest rate of temperature change was observed for the Cryoloop® system, while the lowest one corresponded to the OPS device.

For all the tested heat transfer coefficients the Cryoloop® is the most efficient cooling device system, with a predicted cooling rate of 180000 °C/min for h=1000 W/m^2K. In contrast, the OPS system shows the lowest performance, with a cooling rate of only 5521 °C/min for the same external heat transfer coefficient. Rates of temperature change for the Miniflex® are comparable with the OPS. In Cryotop® system decreasing the drop height from 100 µm to 50 µm improved the cooling rates by 50 % for all the h values.

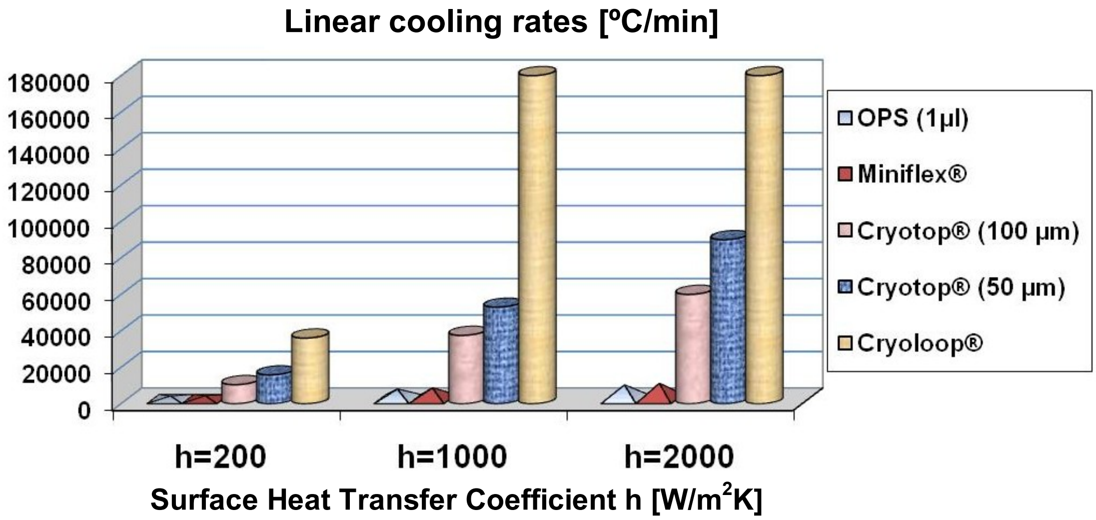

Figure 7. Predicted cooling rates of the different vitrification systems immersed in liquid nitrogen at various external heat transfer coefficients

The core temperature corresponds to the geometrical center of the devices with the exception of the Cryotop®. 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 (P_{hot}) shifts during the process moving towards lower values of z as the h value increases.

For all of the tested 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.

As was noted by He et al. (2008), 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^2K) correspond to heat transfer coefficients for a horizontal thin cylindrical wire plunged in static liquid nitrogen (Kida et al., 1981).

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. 180000 ºC/min) is above the value of 100000 ºC/min which may be considered as ultra-fast cooling (He et al., 2008).

Kuwayama (2007) suggested that the Cryotop® method may produce cooling rates as high as 40000 ºC/min.

Vajta et al. (1998; 2006) indicated that OPS method may render high cooling rates over 20000 ºC/min; however this value is higher than the cooling rates results predicted in our work for the warmest point in OPS (1694 ºC/min for h=200 W/m^{2}K, 5521 ºC/min for h=1000 W/m^{2}K and 7826 ºC/min for h=2000 W/m^{2}K). In contrast when a point in direct contact with LN$_2$ is considered in the OPS system, for example z=0 and r=0 (see Fig. 1 b), the predicted cooling rates are 2244, 10344 and 19148 ºC/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.

5 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 (180000 °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 37500 °C/min for a drop height of 100 μm (Volume=0.0171 μl) and 52941 °C/min for a drop height of 50 μm (Volume= 0.00516 μl),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 numerical simulations and the analysis of the predicted thermal histories could contribute to determine the performance of the different techniques.

6 ACKNOWLEDGMENTS

This study was funded by CONICET, Universidad Nacional de La Plata, Agencia de Promoción Científica y Tecnológica (ANPCYT) and Pontificia Universidad Católica Argentina, Facultad de Ciencias Agrarias, Buenos Aires, Argentina. Authors have expressed no conflict of interest or financial affiliation with any of the commercial cryopreservation devices compared in the study.

REFERENCES

Chian, R.C., Quinn, P., *Fertility Cryopreservation*, Eds.: R.C. Chian, P. Quinn, Cambridge University Press, 2010.

Cremades, N., Sousa, M., Silva, J., Viana, P., Sousa, S., Olivera, C., Texeira da Silva, J., Barros, A., Experimental vitrification of human compacted morulae and early blastocysts using small diameter plastic micropipettes, *Hum. Reprod.* 19:300-305, 2004.

He X., Park, E.Y.H., Fowler, A., Yarmush, L.M., Toner, M., Vitrification by ultra-fast cooling at a low concentration of cryoprotectants in a quartz microcapillary: a study using murine embryonic stem cells. *Cryobiology* 56:223-232, 2008.

Jain, J.K., Paulson, R.P., Oocyte cryopreservation, *Fert. Ster.* 86:1037-1046, 2006.

Kida, M., Kikuchi, Y., Takahashi, O., Michiyoshi, I., Pool-boiling heat transfer in liquid nitrogen, *J. Nucl. Sci, Tech.* 18:501-513, 1981.

Kuwayama, M., Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method, *Theriogenology*, 67:73-80, 2007.

Kuwayama, M., Holm, P., Jacobsen, H., Greve, T., Callesen, H., Successful cryopreservation of porcine embryos by vitrification, *Vet. Rec.* 141:365, 1997.

Luyet, B.J., Hoddap, A., Revival of frog's spermatozoa vitrified in liquid air, *Proc. Meet. Soc. Exp. Biol.* 39: 433–434, 1938.

Luyet, B.J., The vitrification of organic colloids and of protoplasm, *Biodynamica* 1:1–14, 1937.

Mazur, P., Cryobiology: the freezing point of biological systems, *Science* 168: 939-949, 1970.

Mazur, P., Limits to life at low temperatures and at reduced water contents and water activities, *Orig. Life* 10:137–59, 1980.

Rall, W.F., Fahy, G.M., Ice-free cryopreservation of mouse embryos at -196°C by vitrification, *Nature* 313:573–5, 1985.

Sansinena, M., Santos, M.V., Zaritzky, N., Baeza, R., Chirife, J., Theoretical prediction of the effect of heat transfer parameters on cooling rates of liquid filled plastic straws used for cryopreservation of spermatozoa, *CryoLett.* 31:120-129, 2010.

Vajta, G., Holm, P., Kuwayama, M., Booth, P.J., Jacobsen, H. Greve, T., Open pulled straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos, *Mol. Reprod. Dev.* 51:53–58, 1998.

Vajta, G., Kuwayama, M., Improving cryopreservation systems, *Theriogenology* 65:236–244, 2006.