CryoLetters **31** (2), 120-129 (2010)

THEORETICAL PREDICTION OF THE EFFECT OF HEAT TRANSFER PARAMETERS ON COOLING RATES OF LIQUID-FILLED PLASTIC STRAWS USED FOR CRYOPRESERVATION OF SPERMATOZOA

M. Sansinena[1*], M. V. Santos[2], N. Zaritzky [2], R. Baeza[1] and J. Chirife[1]

[1]Facultad de Ciencias Agrarias, Pontificia Universidad Católica Argentina, Cap. Gral. Ramón Freire 183, (1426) Buenos Aires, Argentina.
[2]Facultad de Ingeniería, Universidad Nacional de La Plata and CIDCA (CONICET –UNLP), Argentina
*Corresponding author email: msansinena@fibertel.com.ar

ABSTRACT

Heat transfer plays a key role in cryopreservation of liquid semen in plastic straws. The effect of several parameters on the cooling rate of a liquid-filled polypropylene straw when plunged into liquid nitrogen was investigated using a theoretical model. The geometry of the straw containing the liquid was assimilated as two concentric finite cylinders of different materials: the fluid and the straw; the unsteady-state heat conduction equation for concentric cylinders was numerically solved. Parameters studied include external (convection) heat transfer coefficient (h), the thermal properties of straw manufacturing material and wall thickness. It was concluded that the single most important parameter affecting the cooling rate of a liquid column contained in a straw is the external heat transfer coefficient in LN2. Consequently, in order to attain maximum cooling rates, conditions have to be designed to obtain the highest possible heat transfer coefficient when the plastic straw is plunged in liquid nitrogen.

Keywords: cryopreservation – spermatozoa – heat conduction – thermal diffusivity – straw

INTRODUCTION

The plastic straw was first introduced in Denmark by Sorensen in 1940 for packing liquid semen (25). The first attempts of freezing straws in liquid nitrogen (LN2) vapors were reported in 1961 (1) and later modified by Cassou (6) and Jondet (12). Plastic straws for packing domestic species semen have been the method of choice for the last two decades due to their simplicity, ease of labeling, sealed environment, reduced of storage space requirement and acceptance by professionals for field use (21).

French straws hold volumes of .25, .5 or 1.2 ml, they are typically made of polyvinyl chloride, 135 mm in length and 2.0, 2.8 or 4.2 mm in diameter. One end of the straw is sealed upon contact with liquid with a triple plug which consists of polyvinyl alcohol powder between two cotton plugs. Other types of straws are available;

straws developed in the United States (named "Continental" straws) are made of polypropylene and sealed with two plastic plugs (21, 22). In 1972, Jondet (14) suggested the rapid freezing rate and the geometry of the straw, compared to ampoules, increased the survival of spermatozoa by this method. Moreover, as reported extensively in the literature, higher freezing rates have been associated with higher cell survival by several authors (2). It has been well documented the freezing/thawing process causes extensive damage to spermatozoa. These include membrane integrity damages due to changes in lipid-phase transition and/or increased lipid peroxidation (7, 24), intracellular ice formation (29), altered chromatin structure and DNA damage (11) and all these factors result in reduced fertility and fewer offspring.

Heat transfer plays a key role in freezing rate and efficiency of cryopreservation in plastic straws (18); for this reason modified straws of reduced wall thickness have recently been introduced (31). Because the probability of ice crystal formation is directly proportional to volume and inversely proportional to viscosity and the cooling rate, "open-pulled straws" have recently been developed (23) in an attempt to induce vitrification through increasing heat transfer rates across the wall. Vitrification is a process of converting liquid water into a glass-like amorphous solid without any ice formation. Since water is not very viscous, it can be vitrified only by an extremely rapid cooling of a small sample and/or the introduction of agents that suppress the formation of ice crystals (18). In relation to vitrification in plastic straws, it would be of interest to explore the possibility of using other alternative materials in the manufacturing of semen straws as well as studying the effect of certain heat transfer parameters on the cooling rates.

The aim of the present study is to theoretically estimate the effect of several parameters on the rate of cooling of a liquid-filled polypropylene straw which is plunged directly into liquid nitrogen (LN2). For this purpose the unsteady-state heat conduction equation for concentric cylinders was numerically solved. Parameters studied include the external (convection) heat transfer coefficient (h), the thermal properties of straw manufacturing material and wall thickness.

MATERIALS AND METHODS

Physical Model System:

a) A commercially available polypropylene straw (130 mm length, 2.6 mm o.d.; 1.9 mm i.d.; 0.35 mm wall thickness) was considered as the model system for the heat conduction simulation using the Finite Element Method. The volume of liquid was maintained constant. Therefore for all cases the inner diameter was 1.9 mm ; two values of wall thickness of the polypropylene straw were considered : 0,35 mm and 0,175 mm and its effect on cooling rate was analyzed.
b) Heat transfer through the straw wall and into the liquid column was considered to proceed by conduction; it was assumed (albeit arbitrarily) that vitrification avoided ice formation during cooling.
c) For modeling purposes, two concentric cylinders were considered; the inner cylinder was assumed to be water-filled (see below) and the outer cylinder was the straw manufacturing material. The straw was considered as a finite cylinder.
d) Thermal properties of the spermatozoa suspended in liquid semen extender used under field conditions (19) were assumed equivalent to water properties since water content of the extender is usually 80% or more.

e) The cooling rate (°C/min) is defined as the time needed to reduce initial core temperature of the liquid column from 6 to -90°C while avoiding ice formation.

f) Three external heat transfer coefficients (h=200, 1000 and 2000 W/m^2 K) were used. These were obtained from values reported by Kida et al. (15) for heat transfer from a horizontal wire to saturated liquid nitrogen.

g) The case of an aluminum-manufactured straw (130 mm length, 2.6 mm o.d. ; 1.9 mm i.d.) was also modeled in order to compare the effect of varying the thermal properties of the straw on the cooling rate.

Although accurate computations will require functions of the thermal properties versus temperature, for approximate calculations average values of the thermal properties were used in the temperature range considered (6 to -90°C); values are shown in Table 1.

Table 1. Estimated values of thermal properties of materials used in solving the heat conduction equation

Material (*)	Thermal diffusivity, **α** (m^2/sec)	Thermal conductivity, **k** (W/m K)	Specific heat, **Cp** (J/ kg K)	Density, **ρ** (kg/m^3)
Water	1.1 x 10-7	0.50	4218	983
Polypropylene	1.7 x 10-7	0.22	1680	900
Aluminum	9.9 x 10-5	235	855	2700

(*) Properties correspond to the following temperatures : supercooled water - 23ºC (4); aluminum -23 to -30ºC (8, 9) ; polypropylene 23 - 25ºC (17, 20, 26, 27).

The thermal diffusivity of supercooled water decreases as temperature becomes lower; however between + 5ºC and - 23ºC (28ºC range) it decreases by only 18% (4). Considering that the termal properties of subcooled and vitrified water are yet unknown (10) thermal properties at -23ºC were used in the present work, that is more accurate than using thermal properties of water at 25ºC for the solution as was reported by He et al for heat transfer calculations in a temperature range between 0 to -130ºC (10).

The thermal diffusivities of aluminium and plastic materials in general increase as the temperature decreases but the change is very small; thermal diffusivity of aluminium between +23 and -23ºC increases only by 2.6% (19). For plastic materials, the thermal conductivity of Teflon increases by 15 % in a large temperature range of +23ºC to -73ºC; for polymethylmetacrilate, and polyestirene it increases only about 12 % between +2ºC and -73ºC (30). Therefore, the assumption of a constant thermal diffusivity for polypropylene and aluminium does not appear to introduce an important error in the predictions. As a matter of fact, He et al. (10) in their transfer model to predict cooling rates considered constant thermal properties of capillaries made of various materials.

It is noteworthy thermal properties of different plastic materials may be fairly similar. The thermal diffusivity of polyethylene, polycarbonate and PVC at near room temperature (23-35ºC) are 1.5. 10^{-7}, 1.4.10^{-7} and 1.35.10^{-7} m^2/sec, respectively (5, 8, 16, 28); these values are close to that of polypropylene (see Table 1).

RESULTS AND DISCUSSION

Unsteady-state heat conduction The geometry of the straw containing the liquid was assimilated as two concentric finite cylinders of different materials: the fluid (1) and the straw (2) (Fig. 1) .

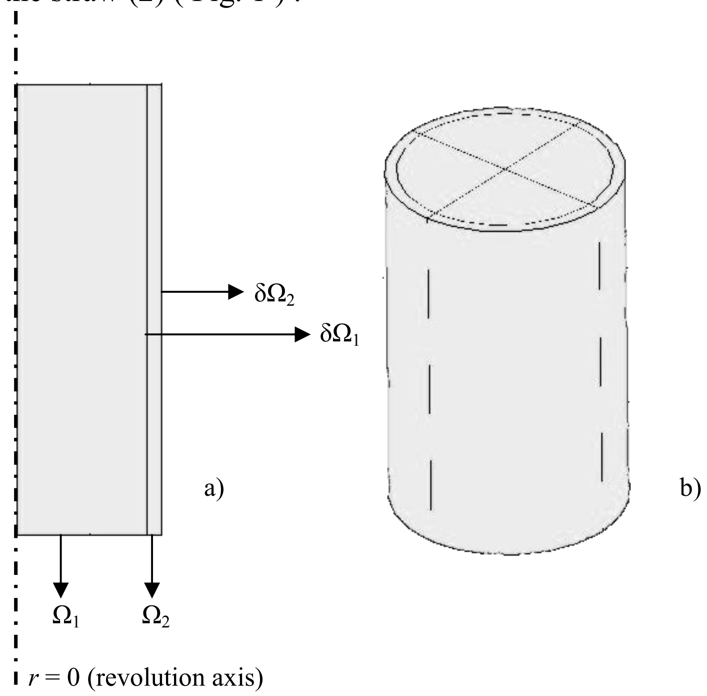

Figure 1. Geometry used for the mathematical model (**a**) Bidimensional revolution surface. (**b**) Three-dimensional revolution solid.

The heat conduction partial differential equations for the axisymmetric problem in cylindrical coordinates for each material is represented as follows:

$$\rho_1\, Cp_1 \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_1\, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_1\, r \frac{\partial T}{\partial z}\right) \qquad \text{in } \Omega_1 \qquad t>0 \qquad (1)$$

$$\rho_2\, Cp_2 \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_2\, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_2\, r \frac{\partial T}{\partial z}\right) \qquad \text{in } \Omega_2 \qquad t>0 \qquad (2)$$

where Ω_1 and y Ω_2 are the domains in which the equations are valid. ρ_i is the density , k_i is the thermal conductivity and Cp_i the specific heat of the material i being i = 1,2, t is the cooling time, r and z are the radial and axial coordinates.

The continuity flux border condition at the interphase $\delta\Omega_1$ between the two materials is :

$$-k_1(\nabla T \cdot n_1) = -k_2(\nabla T \cdot n_2) \qquad \text{in } \delta\Omega_1 \qquad t>0 \qquad (3)$$

where n_1 and n_2 are the external normal unit vectors at the interphase. The equation that represents the boundary convective condition is:

$$-k_2(\nabla T \cdot n_2) = h \cdot (T - T_{ext}) \qquad \text{in } \delta\Omega_2 \qquad t>0$$

(4)

where h is the surface heat transfer coefficient and T_{ext} is the external temperature of the liquid nitrogen and $\delta\Omega_2$ represents the external surface of the straw.

At the symmetry axis the boundary condition is:

$-k_1(\nabla T \cdot n_1) = 0$ in r =0 t>0

(5)

The initial condition was considered uniform in both material domains being:

$T = T_0$ at t = 0 in Ω_1 and Ω_2

(6)

In order to solve the governing heat transfer equations a numerical finite element algorithm was developed to predict the time- temperature curves in both domains. The domains were discretized into elements and point nodes which form the grid structure. The temperature distribution ($\tilde{T}$) at any point of the domain was approximated by using interpolating functions (H) and the node temperatures ($\hat{T}$) in the given element. The temperature was represented by using a finite dimensional space V_h with interpolating functions H (3, 32, 33) being $\tilde{T} = H(x,y,z) \cdot \hat{T}$ (Galerkin Method)

Applying the Green´s theorem the following equation is obtained:

$$\underbrace{\int_{\Omega}(H^t r\rho C_p H d(\Omega))\dot{T}}_{CG} + [\underbrace{\int_{\delta\Omega}(H^t h r H d(\delta\Omega)) + \int_{\Omega}(\nabla H^t \cdot \nabla H)\cdot k r d(\Omega)]}_{KG}\hat{T} - \underbrace{\int_{\delta\Omega} H^t h r T_\infty d(\delta\Omega)}_{FG} = 0 \quad (7)$$

where CG is the global capacitance matrix, KG is the global conductance matrix, and FG the global force vector. $\hat{T}$ is the vector that represents the temperature values at the node points, and $\dot{T}$ represents the $\frac{\partial \hat{T}}{\partial t}$.

$$CG \cdot \dot{T} + KG \cdot T = FG$$

(8)

This semi discrete problem (eq 8) is a system of stiff ordinary differential equations to be solved for the values of T at the nodal points throughout the region. For the time discretization the classical backward Euler method was considered (13). Matlab 6.5 language was used to write the computational program. The code was able to find the temperature changes during cooling of the straw. The z axis was considered in the heat transfer equation in order to be rigorous when describing the mathematical model of the system; however the axial heat transfer effect is almost negligible, due to the very large length/radius ratio.

The temperature ranges selected for this model coincide with landmark temperatures during the process of semen cooling, refrigeration and freezing under field conditions. After a semen sample is obtained and its initial quality parameters are assessed, semen is normally diluted in extender and cooled at an initial rate of 0.67°C/min. Once the sample 6°C it is allowed to equilibrate for 45 min, and subjected to vapor nitrogen freezing and then plunged in liquid nitrogen (-196ºC).

When a straw is plunged directly into liquid nitrogen the following event occurs: if the surface heating the liquid nitrogen is significantly hotter than the liquid, then film boiling will occur where a thin layer of vapor, which has low thermal conductivity, insulates the surface. This condition of a vapor film insulating the surface from the liquid characterizes film boiling. In cryogenic systems knowledge of film boiling is important since the large temperature difference between the object and liquid nitrogen causes film boiling during cooling down from the room temperature (15). Kida et al. (15) conducted an experimental study on heat transfer from an electrically heated horizontal wire to saturated liquid nitrogen at atmospheric pressure. Measured film-boiling heat transfer coefficients for various heater diameters as a function of the excess of the wall temperature above the saturation temperature, were in the range 500 to 1000 W/m^2 ºK.

Figure 2 shows the effect of external heat transfer coefficient (ranging between 200 to 2000 W/m^2K) on temperature change at the midpoint of water-filled polypropylene and aluminum straws immersed in LN2. In all cases, increasing the external heat transfer coefficient increases the rate of temperature decrease; cooling rates for aluminum are always higher than for polypropylene straws due to the large difference between thermal diffusivity of aluminum and polypropylene. At the lowest external heat transfer coefficient (h=200 W/m^2K) the effect of straw material is less significant because rate of cooling is mostly controlled by h. When increasing external heat transfer coefficient (h=1000 and 2000 W/m^2K) heat conduction in the straw material becomes the controlling step and cooling rates become much higher for aluminum than for polypropylene.

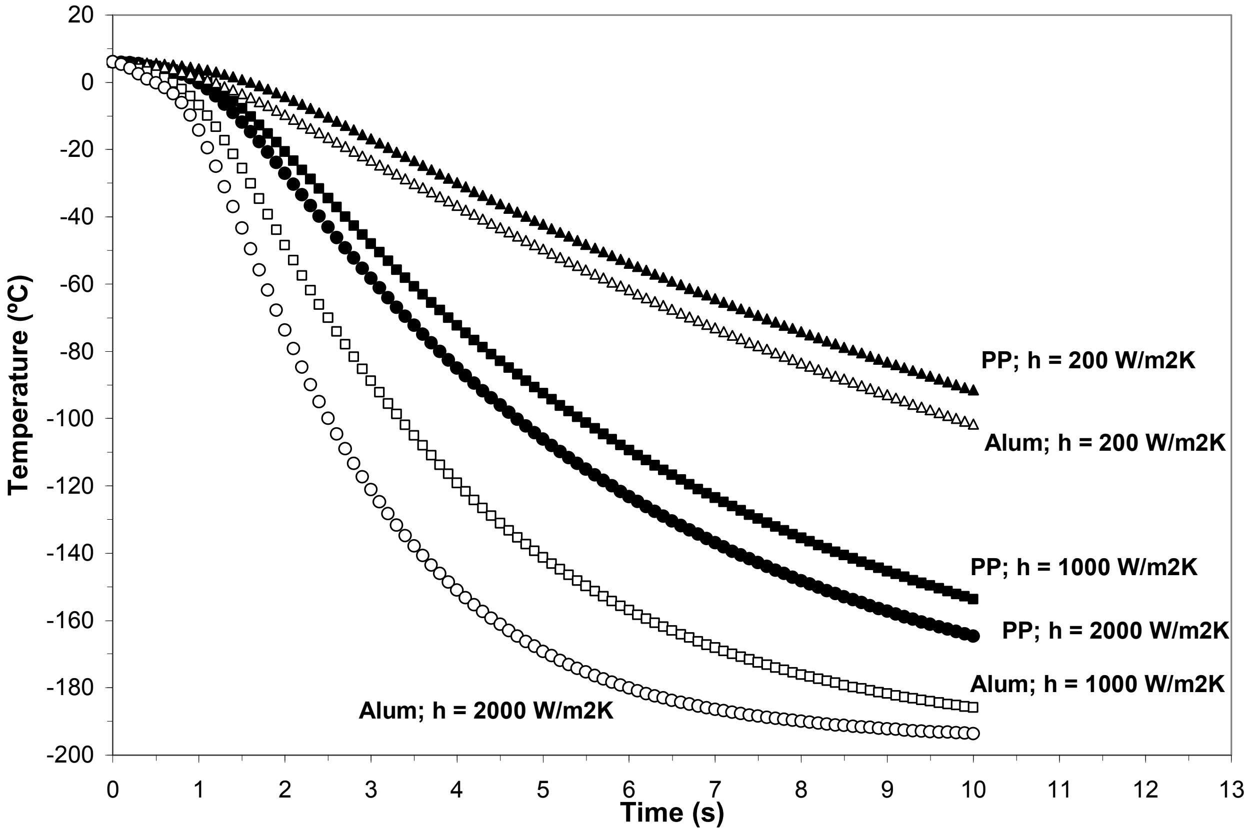

Figure 2. Prediction of the effect of external heat transfer coefficient on rate of temperature decrease at the midpoint of water-filled polypropylene and aluminum straws immersed in liquid nitrogen. PP : polypropylene ; Alum : aluminum.

Figure 3 predicts the effect of reducing wall thickness of polypropylene straws on temperature change upon immersion in LN2. It can be seen that reducing wall thickness from 0.35 to 0.175 mm (while maintining the same internal diameter) increases cooling rates for all external heat transfer coefficients.

This model has shown the reduction of straw wall thickness increases cooling rate. Clearly, the reduction of the volume contained in the straw would also have a similar effect on the cooling rate due to a reduction in total heat conduction path. However, the aplication of this concept to field conditions would require either the modification of the length of the straw to maintain the same semen volume, or to maintain the same length but reducing the insemination volume. Increasing the length of the straw would, in addition, require the modification of semen cryopreservation vessels traditionally used (i.e., the polypropylene straw) and also of the devices used for the delivery of semen into the reproductive tract under field conditions. On the other hand, maintaining the same length would result in reduced insemination doses and this could have negative effects on the expected pregnagncy rates due to low numbers of spermatozoa delivered into the reproductive tract.

Figure 4 combines the results of the effects of external heat transfer coefficient, straw manufacturing material and wall thickness (only for polypropylene) on the cooling rate from 6 to -90°C.

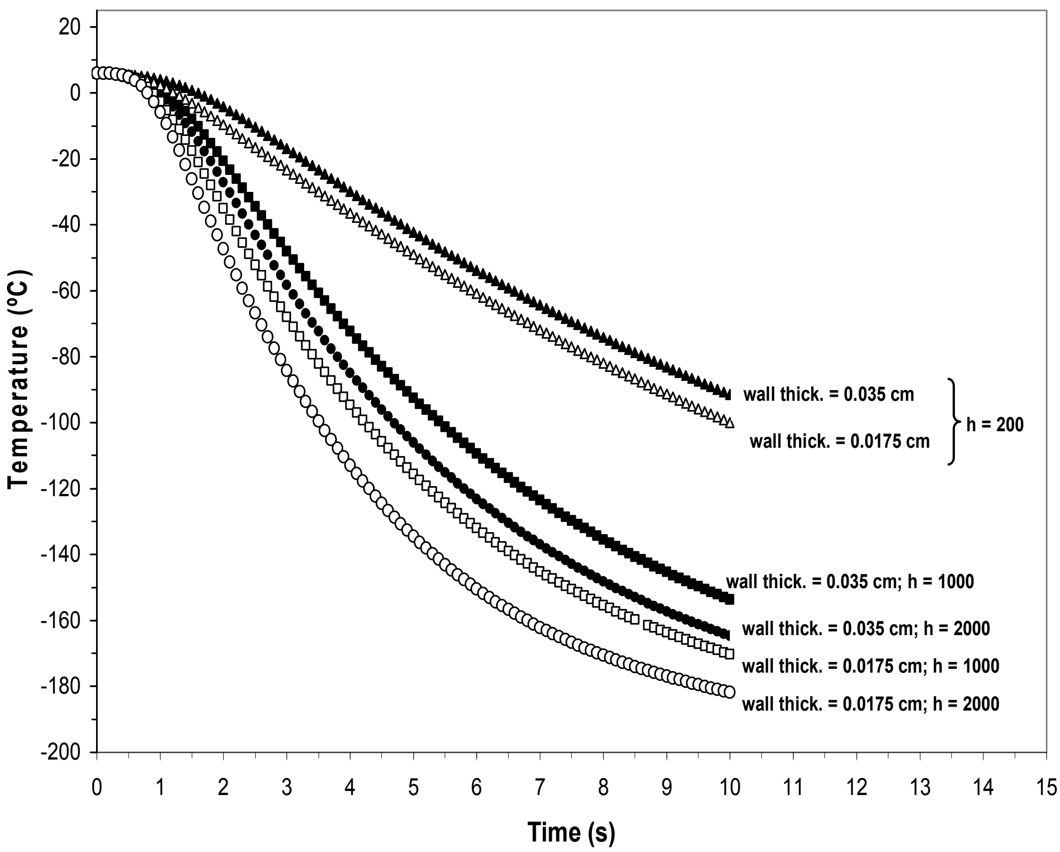

Figure 3. Prediction of the effect of reducing wall thickness of the polypropylene straw on the rate of temperature decrease upon immersion in liquid nitrogen (h is given in $W/m^2 K$).

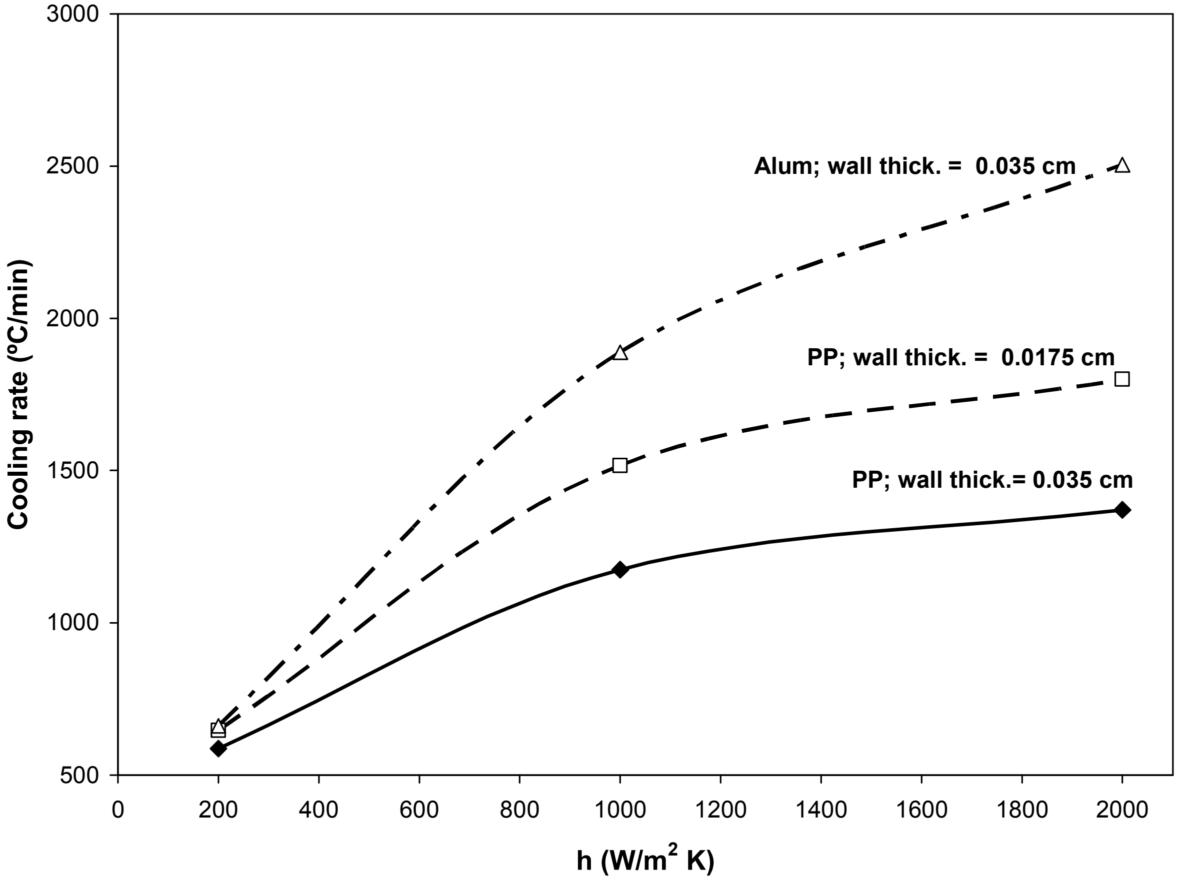

Figure 4. Effect of external heat transfer coefficient on rate of cooling (calculated as time to reduce midpoint temperature of straw from 6 to -90°C when immersed in liquid nitrogen. PP : polypropylene ; Alum : aluminum.

Recently, He et al. (10) studied vitrification of murine embryonic stem cells by ultra-fast cooling in a quartz micro-capillary plunged into liquid nitrogen at a high speed (> 1m/s); capillary dimensions were: outer diameter 0.2 mm, wall thickness 0.01 mm. In their thermal analysis they also used other materials such as the traditional plastic straw (OD = 2 mm, ID = 1.7, wall thickness = 0.15 mm) and the open-pulled plastic straw (OD = 0.95 ; ID = 0.8 mm , wall thickness = 0.075 mm). He et al. (10) developed a thermal model to determine the behavior of the quartz micro-capillary system that are important for achieving ultra-fast cooling. The model accounted for the average convective boiling heat transfer (h) between the external boundaries and liquid nitrogen, which was taken to be 10,000 W. m^{-2} ºC^{-1} . It is noteworthy that their h value is much higher than the range utilized in our model (h ranged between 200 to 2000 W. $m^{-2} K^{-1}$); it was taken from literature data corresponding to heat transfer coefficients for a horizontal thin cylindrical wire plunged in static LN2 (15). This arrangement was considered representative of field conditions for conventional freezing of domestic animal species semen. On the contrary, He et al. (10) used a heat transfer coefficient corresponding to forced convective boiling flow of liquid nitrogen (they plunged the capillary into LN2 at a high speed to create such convective flow of liquid nitrogen). They predicted the effect of boundary boiling heat transfer coefficient on cooling rates and noted it has an affect below a value of approximately 50,000 W.m^{-2}. ºK^{-1}; above that value rate of cooling remained constant for all devices studies (micro-capillary, straws, etc). Our predictions performed for a plastic (or aluminium) traditional straw for packing domestic species semen, showed an important effect of increasing h on cooling rates for the range studied (h 200-2000 $W.m^{-2}. K^{-1}$) in agreement with the previous findings of He. et al. (10). These authors also predicted the effect of capillary dimensions (including inner diameter and wall thickness) and thermal properties of the material used in the devices.

As a whole, our predicted effect of variables - external heat transfer coefficient, wall thickness and thermal properties of the material - on the cooling rates of a conventional plastic (or aluminium) straw plunged in liquid nitrogen, are in general agreement with findings of He at al. (10). However, there is an important difference between their results and the present study. The effect of the aforementioned variables on cooling rates is not straightforward since it also depends on the interaction of controlling steps in the heat transfer in series rom water to straw to LN2. As an example, we found that cooling rates for an aluminium straw are higher than for a polypropylene straw; however at the lowest external heat transfer coefficient, the effect of the straw material is less important because the cooling rate is mostly controlled by h. As the h value increases, heat conduction in the straw material becomes a more significant controlling step being cooling rates much higher for aluminium than for polypropylene, as showed in our Figure 2. Our findings are of direct application to field conditions of domestic species semen cryopreservation, while those of He et al. (10) are related to laboratory ultra-fast cooling conditions. These authors also predicted cooling rates of capillaries made of various materials (i.e. plastic vs. glass, quartz, sapphire, gold, cooper, silver, diamond, etc) having different thermal properties. However, most of those materials are not applicable for straws used in domestic animal semen freezing (and further insemination) due to either, high cost, fragility, or negative effects on sperm viability and fertilizing capacity (cooper).

Regarding the field conditions in cryopreservation of domestic animal semen it is important to note that when a sample is immersed in liquid nitrogen, heat flows from the sample causing liquid nitrogen to boil in the immediate vicinity of the sample creating a pocket of nitrogen vapor around it. Conduction of heat through the nitrogen vapor is much less efficient than conduction of heat through liquid nitrogen since the vapor pocket acts as insulation and retards the heat transfer process.

From our present modeling results it may be concluded that the external (convection) heat transfer coefficient in LN2 is the single most important parameter affecting the cooling rate of a liquid column contained in a straw under field conditions (used for cryopreservation of domestic animal semen). Consequently, in order to attain maximum cooling rates, conditions have to be designed to obtain the highest heat transfer coefficient when the plastic straw is plunged in liquid nitrogen. Also based on the conclusions from the theoretical model, it may be also suggested that the use of a metal with high thermal conductivity properties (i.e. aluminium) in the manufacturing of straw will significantly increase the cooling rate and thus might positively affect sperm viability during cryopreservation.

REFERENCES

1. Adler HC (1961). IV *Inter. Congr. Anim. Reprod. Artif. Insem.* **IV**, 947.
2. Almquist JO & Wiggin HB (1973). *A.I. Digest* **21,** 10-16.
3. Bathe KJ (1996) in *Finite element procedures*. Prentice Hall, New Jersey.
4. Benchikh O, Fournier D & Boccara AC (1985). *J. Physique* **46**, 727-731.
5. Bento AC, Dias DT, Olenka L, Medina AN & Baesso ML (2002). *Braz. J. Phys* **32,** 483-494.

6. Cassou R (1965). *Anim. Breed. Abstr*. **33**, 224.
7. Chatterjee S & Gagnon C (2001). *Mol. Reprod. Dev*. **59**, 451-458.
8. Das K, Kamaruzzaman SM, Middya TR & Datta S (2009). *Indian J. Pure & Applied Physicas* **47**, 273-276.
9. Hatch JE (1984) in *American Society for Metals* (ed) JE Hatch, Metals Park, Ohio, USA. pp 4-24.
10. He X, Park EYH, Fowler A, Yarmush LM & Toner M (2008). *Cryobiology* **56**, 223-232.
11. Hu JH, Li QW, Jiang ZL & Li WY (2008). *Cryobiology* **57**, 257-262.
12. Jondet R (1965). *Anim. Breed. Abstr*.**33**, 227.
13. Johnson C (1987). Numerical Solution of Partial Differential Equations by FEM, Cambridge University Press,
14. Jondet R (1972). VII Inter. Congr. Anim. Reprod. Artif. Insem. **2**, 1348.
15. Kida M, Kikuchi Y, Takahashi O & Michiyoshi I (1981). *Journal of Nuclear Science and Technology* **18,** 501-513
16. Kok M, Demirelli K & Aydogdu Y (2008). *International Journal of Science and Technology* **3,** 37-42.
17. Maier C & Calafut T (1998). William Andrew Publishing, pp 124
18. Mazur P, Cole KW, Hall WH, Schreuders PD & Mahowald AP (1992) *Science* **258**, 1932-1935.
19. Min S, Blumm J & Lindemann A (2007). *Thermochimica Acta* **455**, 46-49.
20. Papa J, Albano C, Bare W, Navarro O, Zannin F & Galarrga D (2002). *European Polymer Journal* **38,** 2109-2117.Pickett BW & Berndtson WE (1974). *J. Dairy Sci*. **57**, 1287-1301.
21. Pickett BW, Berdnston WE & Sullivan JJ (1978). *J. Anim Sci.* **47**, 12-47.
22. Sanchez-Osorio J, Cuello C, Gil MA, Almiñana C, Parrilla I, Caballero I, Garcia EM, Vazquez JM, Roca J & Martinez EA (2008). *Anim Reprod Sci.* **108**, 334-44.
23. Sariozkan S, Bucak MN, Tuncer PB, Ulutas PA & Bilgen A (2009). *Cryobiology* **58**, 134-138.
24. Sorensen E (1940). *Medlemsbldanske Dyrlaegeforen* **23**, 166.
25. Tripathi, D. (2002). iSmithers Rapra Publishing pp 30.
26. Weidenfeller B, Hofer M & Schilling FR (2004). *Applied Science and Manufacturing* **35,** 423-429.
27. Wicaksono H, Zhang X & Fujii M (2001). *The Reports of Institute of Advanced Materials Study, Kyushu University* **15,** 159-163.
28. Woelders H & Chaveiro A (2004). *Cryobiology* **49**, 258-271.
29. Yañez R, Marconi JD, López C, Rubiolo GH & Goyanes SN (2001). *Jornadas SAM-CONAMET-AAS 2001*, 12-14 Septiembre de 2001, Posadas, Argentina.
30. Zhou C, Zhou GB, Zhu SE, Hou YP, Jin F, Zhao XM & Hong QH (2007). *Anim Biotechnol*. **18**, 45-54.
31. Zienkiewicz, OC & Taylor RL (1994). *El método de los elementos finitos*, volumen I. McGraw-Hill, Barcelona.
32. Zienkiewicz, OC & Taylor, RL (1994). *El método de los elementos finitos*, volumen II. McGraw-Hill, Barcelona.

Accepted for publication 18/2/2010