Santos, M. V. ; Sansinena, M. ; Zaritsky, N. ; Chirife, J.

How important are internal temperature gradients in french straws during freezing of bovine sperm in nitrogen vapor?

Documento publicado en:
CryoLetters, 34(2) 2013

CryoLetters **34** (2), 158-165 (2013)

"HOW IMPORTANT ARE INTERNAL TEMPERATURE GRADIENTS IN FRENCH STRAWS DURING FREEZING OF BOVINE SPERM IN NITROGEN VAPOR?"

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

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

Abstract

The subject of present work was to predict internal temperature gradients developed during freezing of bovine sperm diluted in extender, packaged in 0.5 ml French plastic straws and suspended in static liquid nitrogen vapor at -100 ºC. For this purpose, a mathematical heat transfer model previously developed to predict freezing times (phase change was considered) of semen/extender packaged in straw was extended to predict internal temperature gradients during the cooling/freezing process. Results showed maximum temperature differences between the centre and the periphery of semen/extender "liquid" column was 1.5 ºC for an external heat transfer coefficient, h = 15 W/(m^2 K), and only 0.5 ºC for h = 5 W/(m^2 K). It is concluded that if a thermocouple wire were inserted in a 0.5 ml plastic straw to monitor the freezing process in nitrogen vapor, its radial position would have little importance since expected internal gradients may be safely neglected. This finding facilitates the interpretation of freezing rates in 0.5 ml plastic straws immersed in nitrogen vapor over liquid nitrogen, a widely used method for cryopreservation of bovine spermatozoa

Keywords: Freezing - bovine sperm - heat transfer coefficient – temperature gradients - nitrogen vapor - finite element method - French plastic straws.

INTRODUCTION

Semen cryopreservation is a standard practice that permits an efficient utilization and propagation of genetically superior animals and it ultimately leads to overall herd genetic progress (5). The freezing protocol during cryopreservation has a critical impact in sperm survival, viability post-thaw and ultimately conception and calving rates, because cooling rates that are too high or too low can be detrimental for reproductive cells. A widespread practice is to freeze the sample by suspending the straws in static nitrogen vapor (N_2V) over liquid nitrogen (LN_2) for variable periods of time before plunging into LN_2 (-196 °C) for indefinite storage (3). Insulated, Styrofoam® boxes containing LN_2 have been used successfully for sperm cryopreservation. This method can also achieve different cooling rates by changing the distance of sperm samples from liquid nitrogen (13). Different heights/times have reported in literature for freezing of 0.5 ml French straws, with vapor temperatures ranging between -70 ºC to -160 ºC, [5], as recently reviewed by Santos et al. (11).

Recently, Santos et al. (11) developed a mathematical heat transfer model to predict the freezing times (considering phase change) required for bovine semen and extender packaged in 0.5 ml plastic straws and suspended in static liquid nitrogen vapor. Thermo physical properties (i.e. thermal conductivity, specific heat, density, initial freezing temperature) of bovine semen and extender as a function of temperature were determined considering the water change of phase. The non-stationary heat transfer partial differential equations with variable properties (nonlinear mathematical problem) were numerically solved considering in series thermal resistances (semen suspension-straw) and the temperature evolution at the warmest point of the semen/extender packaged in the straw was obtained as a function of cooling time for various N_2V temperatures and external heat transfer coefficients. In this experiment, it was observed in that both the external heat transfer coefficient in stagnant nitrogen vapor and its temperature (controlled by the distance from the surface of liquid nitrogen to the straw) affected freezing times.

Several authors (7, 8) noted that the measurement of the temperature in small cryopreservation vessels during cooling (freezing) is more difficult than anticipated because the thermocouple wire, having a higher thermal conductivity than water, modifies the rate of temperature change within the sample, especially in a small cylindrical objects such as French plastic straws. He noted this effect is present both at the point of measurement and elsewhere, since the wire must pass through half the length of the straw. For this reason, the use of mathematical models to obtain more accurate values of the cooling times was recommended. Katkov et al. (6) also suggested that calculation of cooling rates was a convenient procedure due to difficulties associated with the direct measurement of temperatures in small cryopreservation devices.

Therefore, the objectives of present study were i) to extend the model developed by Santos et al. (11) in order to predict internal temperature gradients in a semen/extender-filled plastic straw when plunged in nitrogen vapor over liquid nitrogen, and ii) to determine whether the controlling thermal resistance in the process is due to the external resistance given by the convective energy transfer (h) or by the internal conduction (k) transfer given by the semen extender solution.

MATERIALS AND METHODS

MATHEMATICAL MODELING

Mathematical Modeling of the Heat Transfer considering Phase Change Transition

The system (straw and semen+extender) can be described as two concentric finite cylinders of different materials: the fluid and the straw. The partial differential equations that represent the heat transfer in the fluid and the plastic support considering radial and axial coordinates are:

$$\rho_s(T)\, Cp_s(T) \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_s(T)\, r \frac{\partial T}{\partial r} \right) + \frac{\partial}{\partial z}\left(k_s(T)\, r \frac{\partial T}{\partial z} \right) \quad \text{in } \Omega s \quad t>0 \qquad \text{(eqn.1)}$$

$$\rho_p\, Cp_p \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_p\, r \frac{\partial T}{\partial r} \right) + \frac{\partial}{\partial z}\left(k_p\, r \frac{\partial T}{\partial z} \right) \quad \text{in } \Omega p \quad t>0 \qquad \text{(eqn.2)}$$

where T is temperature, ρ corresponds to the density, Cp specific heat, k thermal conductivity and the subscripts s and p correspond to the mixture of semen + extender and plastic material, respectively. The phase change transition of water into ice in the semen/extender was taken into account considering the variable thermal properties with temperature ($k_s(T)$, $\rho_s(T)$, $Cp_s(T)$), however in the plastic support the thermophysical properties (k_p, ρ_p , Cp_p) are considered to remain constant. The surfaces exposed to N_2V/LN_2 are the bottom circle and the lateral plastic cylinder; the top circle was considered isolated (q=0) since the semen is in contact with air inside the straw (Figure 1). The warmest point in the system can be identified in the same figure. The equation that represents the boundary convective condition is:

$$-k_p \nabla T.n = h(T - T_v) \qquad \text{in } \delta\Omega_p \qquad t>0 \qquad \text{(eqn.3)}$$

where h is the surface heat transfer coefficient, T_v is the temperature of the N_2V/LN_2 and $\delta\Omega_p$ represents the surface of the straw exposed to the nitrogen vapor. The initial condition was considered uniform in both material domains being:

$$T = T_0 \qquad \text{at } t = 0 \qquad \text{(eqn.4)}$$

Equations (1-4) were numerically solved using the finite element method in COMSOL Multiphysics 3.2. software. The domain was discretized in triangular elements of order 2 in order to obtain accurate numerical approximations. The program calculates the time-temperature curves at all the nodes that constitute the mesh as well as any interior position; in particular at any radial point at the half height of the semen/extender column packed in straw of 1.9 mm internal diameter (see Figure 1), for example Pa, Pb, and Pc.

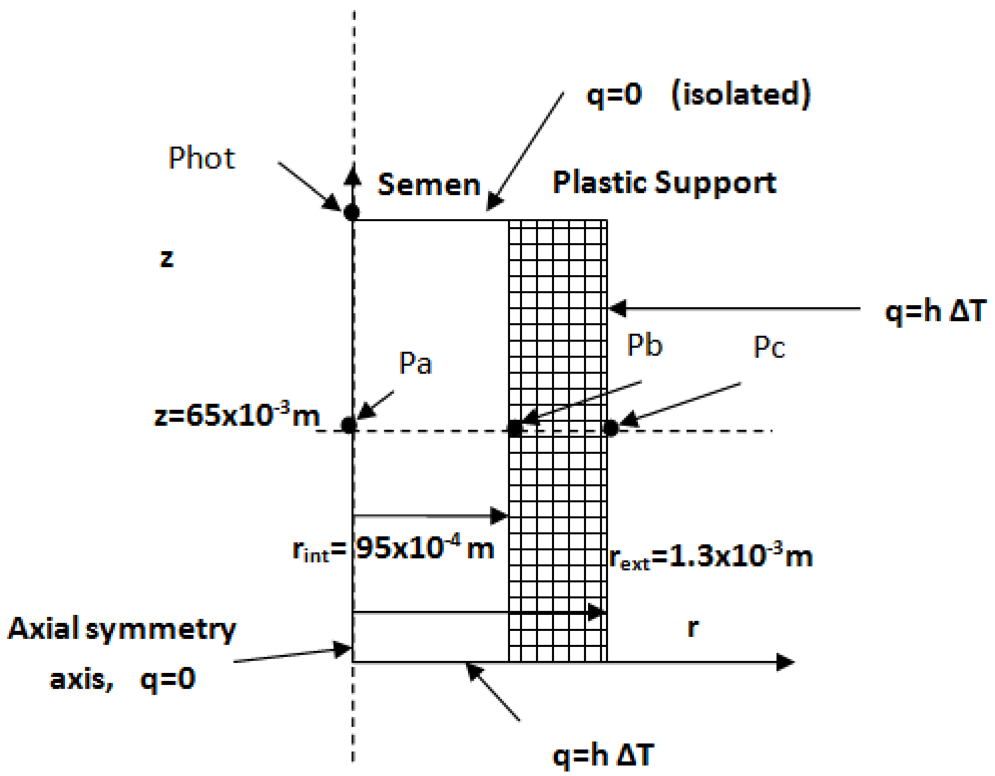


Figure 1. Geometry and dimensions of the straw with visualization of the interior positions, Pa, Pb, and Pc, selected for simulation purposes. Boundary conditions used in the program.

As mentioned before, cryopreservation of sperm is based on freezing of semen in plastic straws (i.e. 0.5 ml French straws) immersed in static N_2V and at a specific distance above LN_2, usually contained in a Styrofoam® box. Since the heat transfer coefficients for this system are not available, literature values for heat transfer coefficients (free convection) in air (78 % nitrogen) were used for the calculations; and two heat transfer coefficient values, h = 5 and h = 15 W/m^2K were used for estimation of the internal temperature profiles.

Thermophysical properties introduced in the model

Ten French polypropylene straws of a commercially available, red Angus bull, were obtained for the characterization of physical/chemical properties of diluted bovine semen. Sperm concentration in 0.5 ml straw was adjusted to 30 x 10^6 and diluted in commercial bull semen extender (Andromed®, Minitube, Germany). Extender composition consisted of phospholipids, TRIS buffer, citric acid, sugars, glycerol, ultrapure water and antibiotics. Dimensions of the polypropylene straw were 130 mm length, 2.6 mm o.d, 1.9 mm i.d. and 0.35 mm wall thickness.

Experimental values and predictive equations that used to determine the thermophysical properties introduced in the model were extensively described in Santos et al. (11). Experimental measurements of thermo-physical properties of bovine semen by Differential Scanning Calorimetry (DSC) include apparent specific heat, unfrozen water content, and latent heat of melting (ΔH_m). In order to overcome numerical instabilities of the program when simulating the heat conduction equation with phase change transition a new mathematical formulation of the apparent specific heat curve was applied using Heaviside and Gaussian functions; this method has been described in detail in Santos et al. (11). Choi and Okos (2) predictive equations were used to obtain the thermal conductivity, density and ice fraction as a function of temperature considering the composition of the semen sample as well as the initial freezing temperature and unfrozen water fraction (11).

RESULTS AND DISCUSSION

Experimental results

The specific heat of the bovine semen+extender (between -150 to 20 ºC) was obtained using the experimental data obtained by DSC. The latent heat of melting was ΔH_m =264.95 KJ/Kg and the unfrozen water resulted in a 4.88% (wet basis). The initial freezing temperature experimentally determined was Tf = - 2.8°C. In order to apply Choi and Okos (2) predictive equations for the thermophysical properties of the semen sample the following composition of the dry matter (mass fractions, wet basis) was adopted: carbohydrates = 0.098, fat = 0.031, and protein = 0.027. The moisture content of the semen+extender was experimentally measured and found to be 84,4 %. The apparent specific heat, thermal conductivity, density and ice fraction vs. temperature were obtained from Santos et al. (11). These data was incorporated in the numerical finite element program to simulate the heat transfer with phase change transition.

Numerical simulations

Few authors have measured internal 0.5 ml straw temperatures during freezing of semen/extender in nitrogen vapor, and determinations of internal gradients were almost unreported in the literature. Chaveiro et al. (1) used cooper-constantan thermocouple wires to monitor “inside” temperature of semen packed in straws (1.6 mm i.d.) but freezing was performed in a freezer cabinet. Shafer-Somi et al. (12) reported the change in temperature inside 0.5 ml plastic straw (radial position not reported) filled with canine semen during freezing in vapor nitrogen. Eriksson and Rodriguez-Martínez (4) measured internal temperatures during freezing of semen packed in Maxi-straws (5 ml); one thermocouple was held in the center and one in the periphery and straws were frozen in a programmable freezing device. Unfortunately the large size of these straws (5 ml) as compared to the 0.5 ml straws, precludes any comparison of data. Therefore considering the limitations on the experimental measurements of internal temperatures for 0.5 ml straws, the numerical program developed by Santos et al. (11) was applied to obtain time-temperature curves in several interior points of the straw. The program allows the prediction of the time-temperature curves at all the nodes that constitute the mesh as well as any specific point by using the interpolating functions. In particular, three points were selected to compare internal temperature gradients: axial position $z = 65\ 10^{-3}$m (half-height of the straw) at three radial positions r = 0 (Pa), $r = 95\times10^{-4}$m (Pb = interface semen-plastic support), and $r = 1.3\times10^{-3}$m (Pc = interface plastic support-N2 vapor). Figure 1 shows the three points (Pa, Pb, and Pc) selected for simulating purposes.

Figure 2 (a,b) shows predicted temperature evolution at different locations in straw during cooling in nitrogen vapor at -100 ºC and for values of the external heat transfer coefficients (h) of 5 W/(m^2 K) and 15 W/(m^2 K).

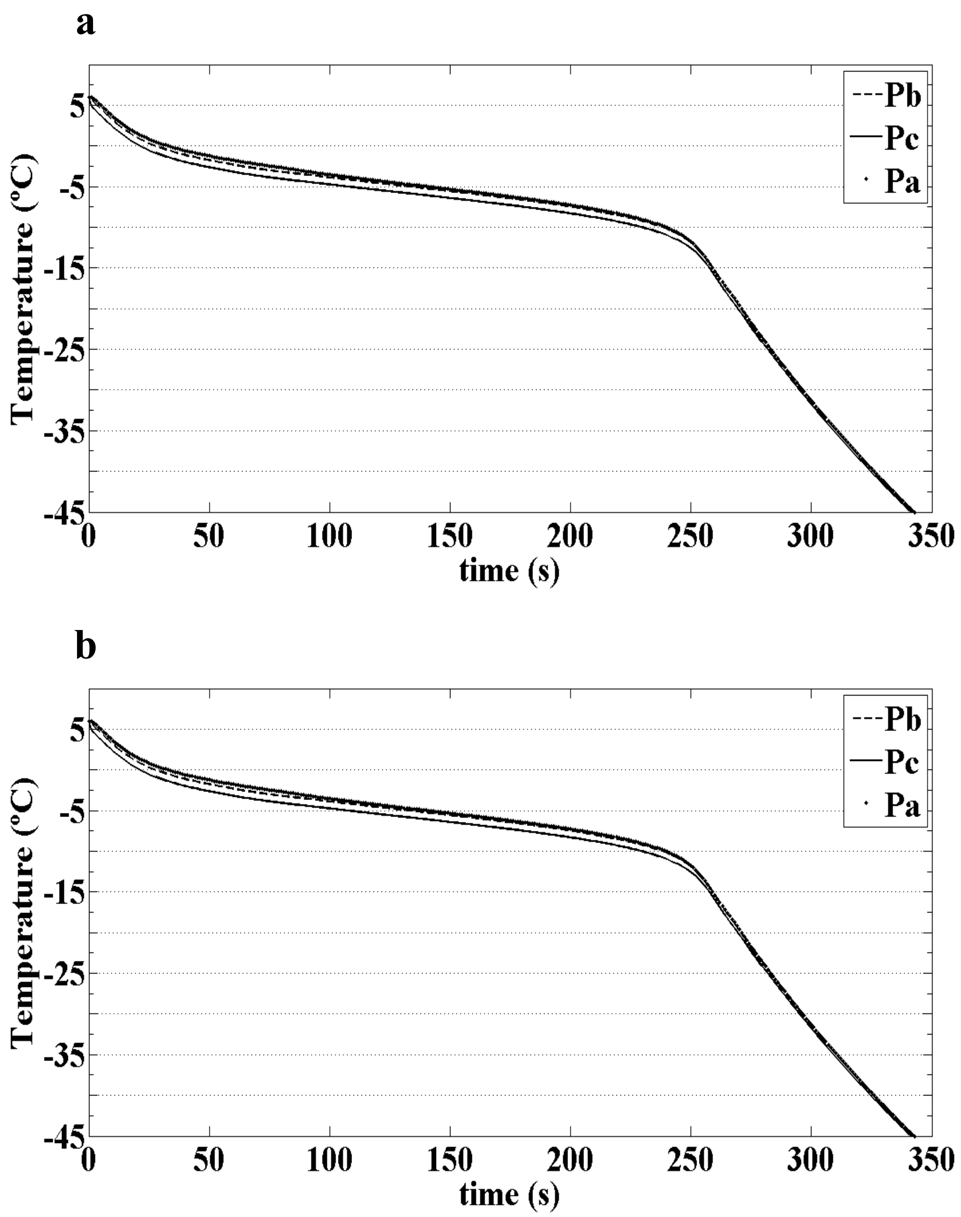
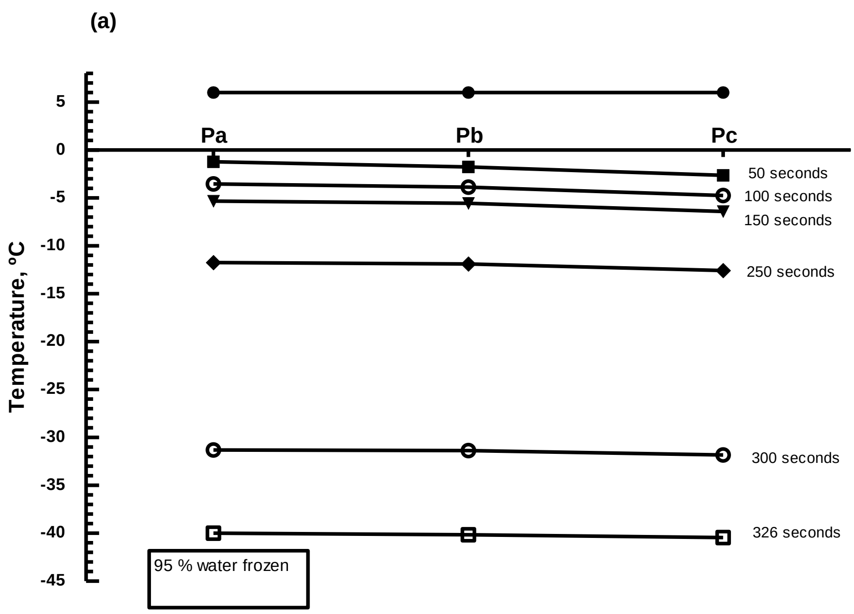


Figure 2. (a) Predicted temperature evolution at different locations in straw (see Figure1) during cooling in nitrogen vapor at -100 ºC and h = 5 W/(m^2 K), (b) Predictive temperature evolution at different locations in straw (see Figure1) during cooling in nitrogen vapor at -100

ºC and h = 15 W/(m^2 K) (for meaning of **Pa**, **Pb** and **Pc**, see Figure 1).

It can be observed that below about -15 ºC, temperature gradients in semen/extender are almost null ; at this temperature (-15 ºC) about 85 % of water has been frozen as indicated by data reported by Santos et al. (11) who determined the ice fraction in bulk semen/extender at various temperatures. The value of the external heat transfer coefficient (h) influences the magnitude of predicted temperature gradients; when h is increased from 5 to 15 W/(m^2 K) thermal gradients are more important. This is explained because at a low value of h heat transfer process is controlled by the external heat transfer, while at higher h internal heat conduction plays a more important role and gradients are more relevant. To better visualize this concept, the Biot numbers were calculated as a function of temperature, being $Biot=h*r_{int}/k_s(T)$ where r_{int} is the internal radius of the straw and $k_s(T)$ is the thermal conductivity of the semen/extender that varies with temperature. From analysis of heat transfer problems in systems with resistances in series, it is known that when Biot number is lower than 1/6 = 0.1667, internal control becomes negligible external control is dominant and no temperature gradients inside the straw are observed. In our cases the minimum and maximum values of Biot were 0.0164-0.0912, for h = 5 W/(m^2 K) and 0.0328- 0.1824 for h = 15 W/(m^2 K). Thermal gradients observed at low values of Biot and specially at h=5 W/(m^2 K) are almost null (0.2-0.5°C).

Figure 3 (a,b) shows temperatures at different locations (Pa, Pb and Pc, Figure 1) in the 0.5 ml straw filled with spermatozoa during freezing in nitrogen vapor at -100 ºC and for different cooling times, up to time at which 95 % of water has been frozen (11). In all cases internal gradients are flattened as cooling time increases. The maximum temperature difference between the center (Pa) and the periphery (Pb) of the semen/extender column is only 0.54 ºC for h = 5 W/(m^2 K) and 1.5 ºC for the case of h = 15 W/(m^2 K).



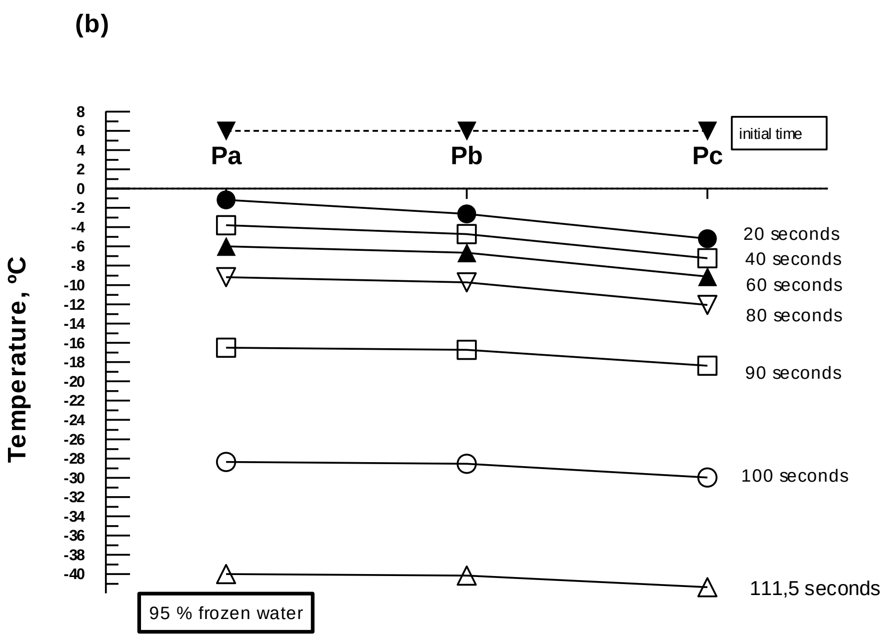


Figure 3. (a) Internal temperature gradients in 0.5 ml straw filled with spermatozoa during

freezing in nitrogen vapor at -100 ºC and h = 5 W/(m^2 K) (for meaning of **Pa**, **Pb** and **Pc** see Figure 1). (b) Internal temperature gradients in 0.5 ml straw filled with spermatozoa uring freezing in nitrogen vapor at -100 ºC and h = 15 W/(m^2 K) (for meaning of **Pa**, **Pb** and **Pc** see Figure 1).

On the other hand and as an example, if the straw is plunged in slush nitrogen the h values in this situation can be over 1000 W/m^2 K [12], the minimum and maximum values of Biot obtained considering h=1000 W/m^2K were 3.27- 18.23, respectively, showing that both external and internal thermal resistances can be relevant.

CONCLUSION

The numerical finite element program allowed the prediction of internal temperatures of bovine semen/extender placed in a 0.5 ml plastic straw and frozen in nitrogen vapor at -100 ºC and the temperature differences between the center and the periphery of the semen/extender column packed in straw of 1.9 mm internal diameter. Results indicate these temperature gradients at low values of h are minimal, amounting to a maximum of 1.5 ºC, and thus may be neglected for practical purposes. This finding, further confirmed by calculation of the Biot numbers that demonstrated an external controlling resistance, facilitates the interpretation of freezing rates in 0.5 ml plastic straws immersed in nitrogen vapor over liquid nitrogen, a widely used method for cryopreservation of bovine sperm.

REFERENCES

1. Chaveiro A, Machado L, Frijters A, Engel B & Woelders H (2006) *Theriogenology* **65,** 1875–1890.
2. Choi Y & Okos MR (1986) in. P. Food Eng. Applic., (ed) Le Magher, Jelen, New York, pp 93-103.
3. Curry MR (2007) *Methods Mol. Biol.* **368,** 303-311.
4. Eriksson BM & Rodriguez-Martinez H (2000) *Animal Reprod. Sci.* **63,** 205-220.
5. Holt WV (2000) *Anim. Reprod. Sci.* **62,** 3-22.
6. Katkov I, Isachenko V & Isachenko E (2009) in: Vitrification in Assisted Reproduction, (eds) Tucker MJ, Liebermann J, Informa Ltd., UK, pp 21-32.
7. Kida M, Kikuchi Y, Takahashi O & Michiyoshi I (1981) *J. Nucl. Sci. Technol.* **18,** 501–13.
8. Mortimer D (2004) *RBM OnLine* **9,** 134-151.
9. Purdy PH (2006) *Anim. Reprod. Sci.* **93,** 114–123.
10. Sansinena M, Santos MV, Zaritzky N & Chirife J (2012) *Theriogenology* **77,** 1717–1721.
11. Santos MV, Sansinena M, Zaritzky N & Chirife J (2012) *Cryobiology*, in press.
12. Schafer-Somi S, Kluger S, Knapp E, Klein D & Aurich C (2006) *Theriogenology* **66,** 173–182.
13. Yasui GS, Arias-Rodriguez L, Fujimoto T & Arai K (2008) *CryoLetters* **29,** 383-390.