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DETERMINATION OF HEAT TRANSFER COEFFICIENTS FOR FRENCH PLASTIC SEMEN STRAW SUSPENDED IN STATIC NITROGEN VAPOR OVER LIQUID NITROGEN

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Abstract

BACKGROUND: The use of mathematical models describing heat transfer during the freezing process is useful for the improvement of cryopreservation protocols. A widespread practice for cryopreservation of spermatozoa of domestic animal species consists of suspending plastic straws in nitrogen vapor before plunging into liquid nitrogen. Knowledge of surface heat transfer coefficient (h) is mandatory for computational modelling; however, h values for nitrogen vapor are not available. **OBJECTIVE:** In the present study, surface heat transfer coefficients for plastic French straws immersed in nitrogen vapor over liquid nitrogen was determined; vertical and horizontal positions were considered. **MATERIALS AND METHODS:** Heat transfer coefficients were determined from the measurement of time-temperature curves and from numerical solution of heat transfer partial differential equation under transient conditions using finite elements. The h values experimentally obtained for horizontal and vertically placed straws were compared to those calculated using correlations based on the Nusselt number for natural convection. **RESULTS:** For horizontal straws the average obtained value was h=12.5 ± 1.2 W/m^2 K and in the case of vertical straws h=16 ± 2.48 W/m^2 K. The numerical simulation validated against experimental measurements, combined with accurate h values provides a reliable tool for the prediction of freezing curves of semen-filled straws immersed in nitrogen vapor. **CONCLUSION:** The present study contributes to the understanding of the cryopreservation techniques for sperm freezing based on engineering concepts, improving the cooling protocols and the manipulation of the straws.

Keywords: plastic French straw, heat transfer coefficient, nitrogen vapor, mathematical model, cryopreservation, freezing

INTRODUCTION

It is well known that cryopreservation of mammalian spermatozoa is a fundamental step in the conservation of valuable genetics, improvement of genetic progress and the application of assisted reproductive technologies (14, 20). Cryopreservation of semen is mostly done using slow cooling protocols, mainly because it allows to store the relatively large volumes of diluted ejaculate (from 0.25 to 0.5 ml) necessary for artificial insemination in domestic animal species (1, 11, 14, 23). The freezing procedure is a critical factor on sperm viability, because cooling rates that are too high or too low can be detrimental to cell viability. Rapid cooling causes a shortage in the period of water efflux from the cell, resulting in excessive intracellular ice formation and consequent cell death (17, 18, 24). On the contrary, slow cooling often injures the cells due to mechanical and/or osmotic effects and cryotoxicity caused by prolonged exposure to the external medium (16, 17). Automated, programmable freezers are set to accomplish controlled, slow cooling of bovine semen packed in polypropylene straws (8, 9, 31). In field conditions, however, cryopreservation is commonly achieved by horizontally suspending the straws in nitrogen vapor over liquid nitrogen (N_2V/LN_2) for variable time intervals before plunging into LN_2 (-196ºC) for a prolonged storage (9). A widespread practice is to freeze sperm-filled French straws in insulated containers horizontally suspended in N_2V/LN_2; various combinations of freezing heights (hence different N_2 vapor temperatures), and times have been reported with acceptable results in terms of cell viability post-thaw (4). Another freezing procedure reported by Takeo and Nakagata (30) consists of placing semen-filled French straws in vertical position in nitrogen vapors at the neck of a Dewar tank. When plastic French straws are placed in N2V/LN2, heat transfer by natural convection occurs, since nitrogen vapors (N_2V) are heated by the sample changing their density and causing movement of the surrounding fluid. This velocity due to the change of nitrogen vapor density is less marked at the zone between the straw and the cold source (liquid nitrogen) below it; in this case the low temperature vapors having a higher density will settle close to the bottom or near the liquid nitrogen where stratification phenomenon occurs. On the other hand, in the zone above the straw, which is the hot surface that is facing upward, nitrogen vapors in contact with the sample have less density and tend to rise upward. Buoyancy forces lead to natural convection rates that depend upon the physical constants of the fluid such as: density, viscosity, thermal conductivity, specific heat and coefficient of thermal expansion β (beta) which for gases = 1/T[K]. Other factors that also affect convection-heat transfer coefficients (h) are: the characteristic dimension of the system and the temperature difference between the cold and hot surfaces (ΔT). In order to correctly assess and predict the temperature of straws during the freezing process one of the main parameters that must be determined is the surface heat transfer coefficient (**h**). The **h** value is present in the natural convective boundary condition of the partial differential heat transfer equation; it depends on the shape and relative position of the object, the fluid-dynamic behavior of the vapors around the solid, and the roughness of the object surface (2, 12). The temperature distribution in the vapor phase at different heights over the liquid nitrogen therefore depends greatly on the distance between the sample and liquid nitrogen surface, and on the velocity field of the N_2vapor (25). The heat transfer coefficient (**h**) and the N2 vapor temperatures are crucial factors to be determined in order to correctly implement freezing protocols that will achieve sperm survival. To date, there is scarce information about experimental determination of heat transfer coefficients for plastic French straws during freezing in N2 vapor. There is also lack of information regarding the influence of the nitrogen vapor temperature profile developed above the liquid nitrogen interface. Santos et al. (27, 28) described a mathematical model to predict actual freezing times required for bull spermatozoa/extender packaged in polypropylene straw to determine ‘‘safe’’ holding times of plastic straw in static nitrogen. In the absence of actual values for **h** in N_2V over LN_2 literature values of heat transfer coefficients (free convection) in air (78% nitrogen) were used for the calculation of freezing times of plastic straws immersed in static N_2V at specific distances above LN_2. Recently, experimental determination of **h** values in plastic French straws plunged in LN_2 were determined (26). However, experimental values of **h** for straws suspended in N_2V/LN_2 have not yet been reported. The general objective of the present study was to determine heat transfer coefficients for plastic French straws, adopting vertical or horizontal positions and suspended in static nitrogen vapor over liquid nitrogen (N2V/LN2). The specific objectives were: i) to experimentally measure the time- temperature curves of straws containing ice, or bovine semen-extender during freezing in N2V/LN2, ii) to apply a numerical finite element program in order to solve the heat transfer partial differential equation with convective boundary condition at the interface plastic-N2 vapor during cooling, to obtain temperature profiles; iii) to determine the heat transfer coefficients that govern the cooling process using experimental and numerical results, and iv) to compare the obtained **h** values with those calculated by using published correlations based on the dimensionless Nusselt number.

MATERIALS AND METHODS

Experimental setup

Experiments were carried out using straws filled with: a) ice, or b) bovine semen-extender. Ice was selected to conduct experiments, since it does not suffer phase change transition in the studied temperature range and their thermo-physical properties are well known (3, 4). As opposed to ice, bovine semen-extender system undergoes phase transition and freezing upon cooling. Cylindrical plastic straws used for cryopreservation of bovine semen were obtained from AB Technology, Inc. (Pullman, Washington, USA). The average external diameters, length and thickness were D=2.81mm, L=124mm, e=0.21mm, respectively. Straws were filled with ultra-pure, reverse-osmosis filtered water (Milli-Q, Milipore Corporation, MA, USA) that freezes at 0 ± 0.3ºC. For semen-filled straws, semen was obtained from adult, Red Angus bulls of proven fertility and good body condition. Ejaculates were diluted in a commercial tris-buffered extender (Triladyl, Minitube®, Germany) containing egg yolk (20% v/v) and glycerol (6% v/v) and adjusted to a final insemination dose of 10 $x10^6$ sperm/straw. The time-temperature curve was recorded using a thermocouple type T (Copper-Constantan) inserted in the central axis of the straw. The thermocouple was connected to an acquisition device (TESTO, Germany). In order to avoid radial drift of the thermocouple it was threaded into a 20-G needle as a vertical guide. The outside diameter of thermocouple junction was 0.22 mm. To record temperature-time data during cooling, ice filled straws were first maintained at a constant temperature ranging between -15 to -19ºC in a conventional freezer. The semen filled straws were maintained at 4-5°C in all the experiments before cooling in LN2/N2V. The straws were then rapidly immersed in nitrogen vapor (N_2V) over liquid nitrogen (LN_2) contained in a Styrofoam box (height 23 cm, length 38 cm and wide 21 cm) and placed at two different heights (2.5 ± 0.5 cm and 12± 0.5 cm) measured with reference to the level of LN2. Another set of experiments were carried out suspending the straws with semen samples in nitrogen vapors at the neck of a Dewar tank. The vapor temperature at each position was also determined using thermocouples. All the experiments were carried out in triplicates.

Temperature measurement in horizontally positioned straws

Since the mathematical formulation for a biological fluid such as semen undergoing phase change transition constitutes a complex mathematical problem due to the abrupt changes in the thermophysical properties (6, 10, 29), a simpler system (ice-filled straw) was first chosen to calculate the range of values of the surface heat transfer coefficients. Once the **h** values were calculated using ice, they were then applied to predict the cooling curve of straws filled with bovine semen immersed in LN2/N2V. The protocol commonly used to freeze under field conditions was used; it consisted in suspending the straw during 3 minutes in N_2V at a height of 12cm followed by 7min at 2.5cm using as reference the level of liquid nitrogen. The temperature distribution inside the box was recorded placing thermocouples at different heights above the liquid nitrogen. Figure 1 shows the experimental setup for the experiments.

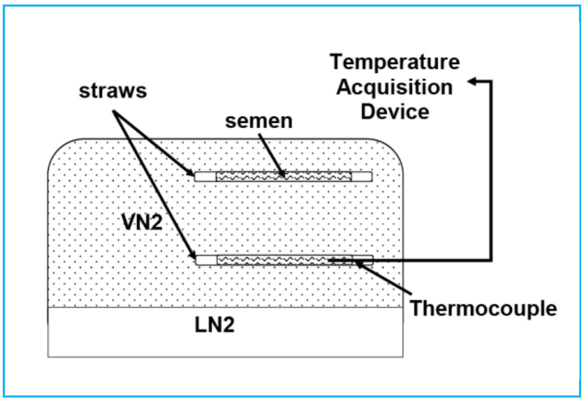

Figure 1. Schematic diagram of the experimental setup for freezing of straws with semen in a Styrofoam box containing liquid nitrogen and nitrogen vapors.

Temperature measurement in vertically positioned straw

Semen-filled straws were placed in the Dewar canister in a vertical position and then suspended near the neck tube of the tank avoiding immersion in the LN2. The base of the canisters has small bottom holes allowing nitrogen vapors to rise surrounding the straw and cooling the sample. It must be taken into account that the nitrogen vapor temperature varies along the neck tube, and it was also measured during the experiment.

Mathematical modeling

The system (plastic-filled straw) can be described as two concentric finite cylinders of different substances: the inner material being either ice or semen and the outer the plastic straw. The partial differential equations that represent the heat transfer in the fluid that is submitted to the freezing process (Eq. 1) and in the plastic support (Eq. 2) considering radial and axial coordinates have been thoroughly described in Santos et al. (27, 28), and are as follows:

(1)

$$\rho_s(T)\, Cp_s(T) \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_s(T)\, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_s(T)\, r \frac{\partial T}{\partial z}\right)$$

(2)

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

where: T is temperature, ρ is the density, Cp specific heat, k thermal conductivity. The subscript s corresponds to the inner material (ice or biological fluid) and p to the plastic material. In the case of the plastic support the thermophysical properties (k_p, ρ_p ,Cp_p) are considered constant. In contrast the inner material has temperature dependent thermal properties; in the case of ice the phase change transition was not included in the studied temperature range, however for the biological fluid that undergoes freezing, thermo-physical properties change markedly with temperature leading to a highly non-linear mathematical problem (27, 28). The initial temperature condition was considered uniform in both material domains.

The convective boundary condition at the interface plastic support and nitrogen vapor (VN2) is:

(3)

$$-k_p \nabla T.n = h(T_{wall} - T_{ext})$$

where h is the surface heat transfer coefficient, k_p is the plastic thermal conductivity, T_{wall} is the variable surface wall temperature of the interface plastic support-VN2, T_{ext} is the external temperature of VN2, **n** is the normal outward vector, and ∇T is the temperature gradient evaluated at the surface. The numerical program calculates the temperature profile as a function of time, in the straw and in the semen during the freezing process. Different heat transfer coefficients were introduced to simulate the temperature-time curves for straws; experimental and predicted temperatures for each h value were compared. The heat transfer coefficient that minimized the Residual Sum of Squares given by Eq. was selected.

(4)

$$RSS = \sum (T_{exp} - T_{pred})^2$$

The ice content as a function temperature was estimated using the equation proposed by Miles et al. (1983):

(5)

$$xh = (x_{wo} - xb)(1 - \frac{Tf}{T})$$

Where xh is the mass fraction of ice, Tf is the initial freezing temperature (Tf = -2.8°C), and T is the temperature at a specific radial and axial position and time, both are given in ºC, x_{w0} is the total mass fraction of water in the sample (x_{w0}=84.4%, wet basis). The value xb represents the unfreezable water present, xb= 4.88% (wet basis), the value was experimentally measured using Differential Scanning Calorimetry, as explained in Santos et al. (27).

Thermophysical properties

In the case of ice-filled straws the thermo-physical properties used as input in the numerical program were as follows: the average specific heat was considered 1461.7 J/kgºC for temperatures below 0ºC. The thermal conductivity and density of ice as a function of temperature were obtained from literature (3, 4,). The thermo-physical properties of the semen+extender were calculated based on the composition of the biological fluid expressed in wet basis mass fraction: 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%. Choi and Okos (3) equations were applied to estimate the thermal conductivity and density. Specific heat and latent heat of ice melting of the semen + extender mixture were measured by using a Differential Scanning Calorimeter (DSC) (TA Instruments, New Castle, Delaware, USA) model Q100 controlled by a TA 5000 module. Experimental data of the apparent specific heat, where the sensible heat is merged with the latent heat, produced a curve with a large peak around the freezing point. However for mathematical modeling purposes the specific heat capacity function has to rise smoothly to a peak over a finite range of temperature (22). Therefore a reformulation of the Cp based on experimental data of Cp vs. Temperature was constructed using a Gaussian and Heaviside function (22) considering the experimental values of the initial freezing point, the range of temperature change and the latent heat of melting. The equation used in the numerical program to represent the specific heat as a function of temperature is as follows:

(6)

$$Cp(T) = Cp_{ff} + \frac{\Delta H_m}{Ts} \quad f(H_{ea}) + D\,\Delta H_m$$

Where Cp_{ff} is the specific heat of the fully frozen state, Ts is the peak temperature point and D is a gaussian curve defined as

(7)

$$D(T) = \frac{e^{\left(\frac{-(T-T_m)^2}{dT^2}\right)}}{\sqrt{\pi dT^2}}$$

where dT is the half width of transition, that is the temperature difference from melt within which 84% of the latent heat occurs; f(Hea) is the Heaviside function which is a built in function in COMSOL Matlab environment that has continuous second order derivatives. This function enables the numerical finite element software (Comsol AB Multiphysics, 2005) to successfully deal with the abrupt change in the apparent specific heat of the sample with temperature avoiding numerical instabilities or divergence of the solution. The specific heat of the biological fluid obtained by DSC and using Eq. 6-7 can be observed in Figure 2.

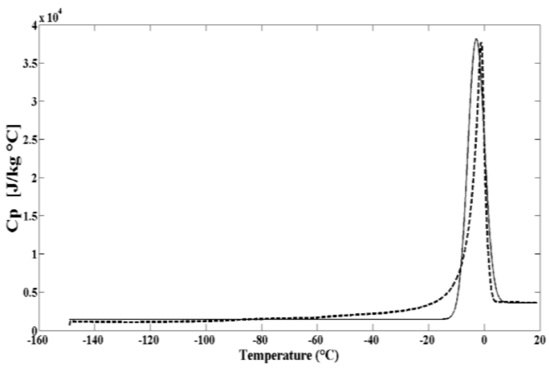

Figure 2. Apparent specific heat of semen+extender sample experimentally obtained by DSC (- -) and using the Heaviside and Gaussian functions (▬)

RESULTS AND DISCUSSION

Surface heat transfer coefficient for horizontally positioned straws

Figure 3 (a, b and c) shows examples of measured and calculated time-temperatures for ice-filled straw at different distances (hence different temperatures) from LN2. At a distance of 12 cm the external nitrogen vapor temperature was -63.9ºC and an excellent agreement between the experimental values and the temperature predictions (using the numerical program) was found for a h= 10 $W/m^{2o}C$. (Figure 3 b) shows another experiment at a 2.5 cm height with an external nitrogen vapor temperature of -119ºC; the obtained h value was 12 $W/m^{2o}C$. Figure 3c shows another experiment at 12 cm from LN_2 and an external temperature of -114°**C**; the calculated h value was h=14 W/m^2 K. The computational program was afterwards applied to simulate the freezing process of semen filled straws using the freezing protocol previously described i.e., suspending the straw during 3 minutes in N_2V at a height of 12cm followed by 7 min at 2.5 cm using as reference the level of liquid nitrogen.

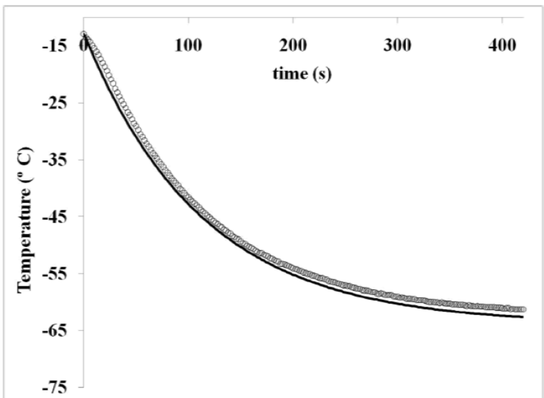

Figure 3a. Experimental temperature (o) and Numerical Predictions (▬) for plastic French straws with ice. T_{ext}= -63.9ºC ,T_i=-12.9ºC, h=10 W/m^2 C, height =12cmfrom the level of liquid nitrogen. (Styrofoam box with lid).

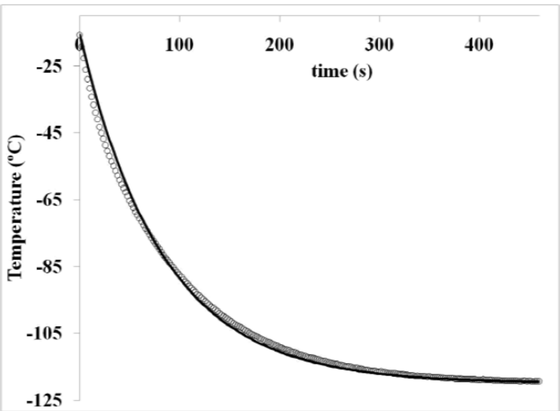

Figure 3b. T_{ext}= -119ºC ,T_i=- 17.9ºC, h=12 W/m^2 C, height =2.5cm. (Styrofoam box with lid).

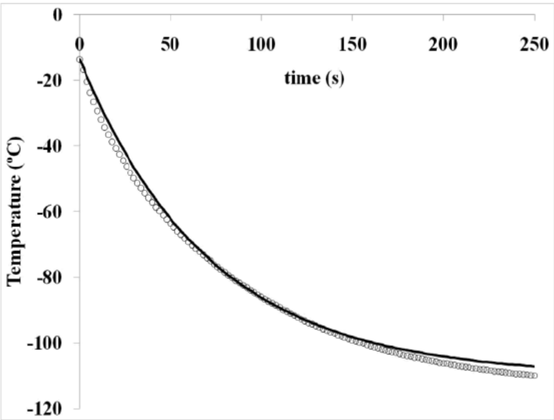

Figure 3c. Experimental temperature (o) and numerical predictions (▬) for plastic French straws with ice. T_{ext}= -114ºC ,T_i=-13.6ºC, h=14 W/m2 C, height =12cm. Styrofoam box without lid.

Figure 4 shows an example of the time-temperature measurements and the numerical predictions for the semen filled straw; an excellent agreement between the data using an h value of 13 W/m^{2o}C was observed. As semen undergoes freezing the typical plateau region was observed. The average h value obtained in all the experiments (with or without lid and at different heights) was h=12.5 ± 1.2 W/m^2 K (confidence interval 95%). The h value was independent of the height position of the straw over the level of liquid nitrogen. This result is in agreement with the literature reports (2, 12, 13) since the heat transfer coefficient is not a material property; it is a parameter that reflects the mechanisms in which a particular surface and fluid interact when they come in contact. Additionally the presence of the box lid was not significant (P>0.05) on the measured h values.

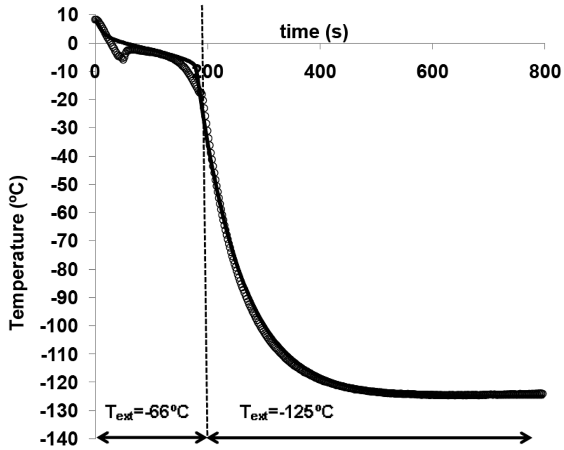

Figure 4. Experimental temperature (o) and Numerical Predictions (▬) for plastic French straws containing semen+extender. T_i=8.3ºC, h=13 W/m^2 C, Cooling protocol:3 min at height 12cm (T_{ext}= -66ºC) and then 7 min at 2.5 cm (T_{ext}=-125ºC), (Styrofoam box with lid).

Surface Heat Transfer Coefficient for vertically positioned straws

Figures 5 a and b show the experimental time–temperature data in straws placed vertically in canisters at the neck tube of the Dewar flask and the numerical predictions using the computational code that considers the phase change transition during freezing of the semen+extender. Good agreement between curves can be observed, indicating coinciding theoretical versus experimentally determined h. The average surface heat transfer coefficient and the confidence interval (95%) obtained for straws suspended vertically in nitrogen vapor over liquid nitrogen was 16 ± 2.48 W/m^2 C. The vertical position of the straw in the neck tube causes the bottom to be submitted to a lower N2V temperature and then a higher freezing rate than the top part of the straw (total length 12cm). These conditions produce a non-uniform ice fraction distribution in straw along the axial direction. This does not occur in the horizontally placed straws since the temperature of the nitrogen vapors depend on the height over the level of LN2. Sansinena et al. (25) described the temperatures of nitrogen vapors in Dewar tanks at different heights over liquid nitrogen under various conditions (full, half full tank, in stagnant and after moving the canisters in the neck tube). In order to visualize the effect of the external temperature profile in the nitrogen vapor on the freezing curve of the semen filled vertical straw numerical simulations were carried out. Temperature profiles and ice formed fraction in a straw located in a half full Dewar tank, were simulated at the bottom and the top part of a straw, where the measured external N2V temperatures were -189.56ºC and -44ºC, at 22 cm and 10 cm distance from the top respectively.

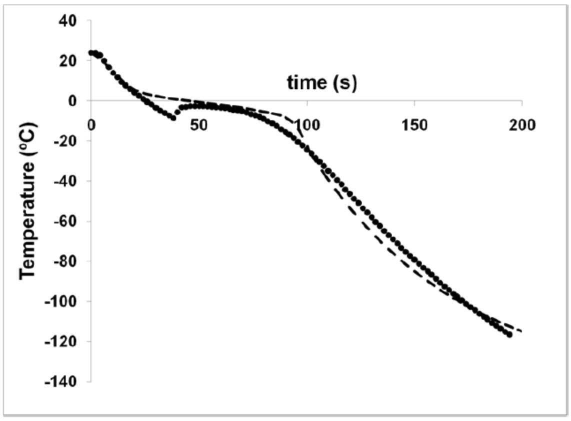

Figure 5a. Time temperature data (●) and numerical predictions (- -) for plastic French straws filled with semen+extender placed vertically in nitrogen vapor over liquid nitrogen. Text= - 143ºC, h=15W/m^{2}ºC, Ti=24.1ºC.

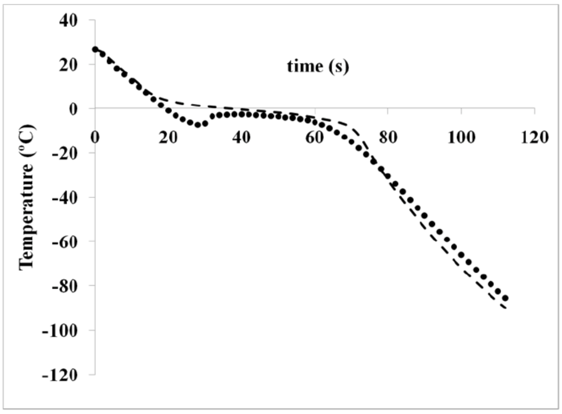

Figure 5b. Straw placed vertically in the neck tube. Text= -173ºC, h=17 W/m2ºC, Ti=26.1ºC.

Figures 6a and b. show the simulated freezing experiment and the numerical results.

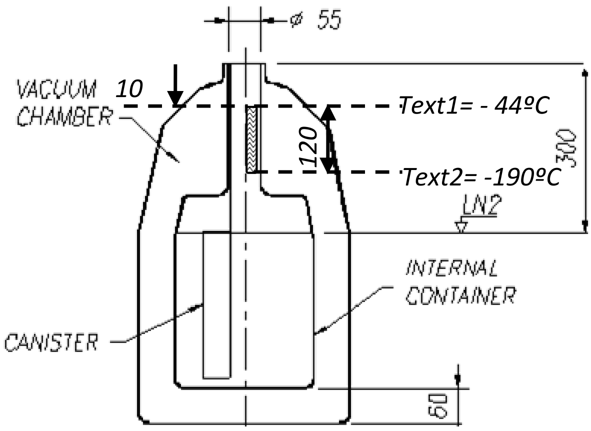

Figure 6a. Schematic diagram of the freezing experiment in the Dewar flask. The top of the straw (L= 12cm) is located in the neck tube.

It can be observed how the different external nitrogen vapor temperatures affected the cooling rate and therefore the ice fraction as the freezing process evolved.

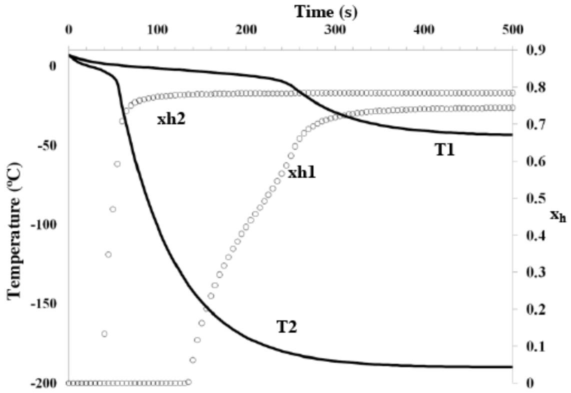

Figure 6b. Numerical temperature predictions **T1 and T2** in the straw (▬) and corresponding ice fractions **xh1, xh2** (o) vs. time for nitrogen vapor temperatures Text2 = - 44ºC (at the top of the straw) and Text2=-190ºC (at the bottom of the straw) respectively. The average h=16 W/m^2 K.

This demonstrates a non-homogeneous ice fraction distribution in a vertically frozen straw. It must be pointed out that the h value used in the simulations of T1 and T2 was maintained constant (h=16 W/m^2 K), since this parameter reveals the fluid-dynamic behavior and how nitrogen vapors interact with the straw. The h value does not depend on the height above the level of liquid nitrogen, or the external nitrogen vapor temperature.

Correlations for Surface Heat Transfer Coefficient

The h values were further compared with literature correlations for natural convection in cylinders using the dimensionless Nusselt number for vertical and horizontal configurations. The following dimensionless numbers were calculated for natural convection (Grashof)

$$Gr = \frac{gD^3(\rho_f)^2 \Delta T(\beta_f)}{(\mu_f)^2}$$

where D is the diameter of the straw, g the gravitational constant, ρ the density and β the compressibility factor (for ideal gases is $1/T_f$), μ is the viscosity, $\Delta T = |T_0 - T_\infty|$ where T_0 is the temperature of the straws surface and T_∞ is the temperature of nitrogen vapor away from the object. The Prandtl number

$$Pr = \frac{Cp\, \mu_f}{k_f}$$

where Cp is the specific heat capacity and the subscripts f corresponds to the properties of the nitrogen vapor evaluated at the temperature $T_f=(T_0+T_\infty)/2$. For horizontal heated cylinders under natural convection Mc Adams (19) suggested the

following correlation, $Nu=0.53\ (Gr\ Pr)^{1/4}$ for the range $10^4<Gr\ Pr<10^9$. However, in our case the range of GrPr values was lower (150<Gr.Pr<350) and according to the correlation the Nusselt correlation led to an average value of h= 16.7 W/m^2 K. This value is somewhat higher than the experimentally obtained h for horizontal configuration and this can be attributed to the stratification of the nitrogen vapors in the Styrofoam box. During cooling of straws the cylinder represents the hot surface located at the top of the cooling box and the liquid nitrogen interface (cold source) is at the bottom. It is known that hot surfaces facing down produces a stratification phenomenon of the fluid (nitrogen vapor). In a normal fluid for which the density decreases with increasing temperature, the temperature field creates a situation in which less dense layers are located above denser ones, a stable situation which does not cause any convection currents; heat is transported by conduction only and the heat transfer coefficients decrease significantly in comparison to the situation where the cold source is facing down or a hot source is facing up (7). For vertical configuration the h values were initially calculated using the correlation of Churchill and Chu (1975) for vertical plates. However for vertical cylinders such as the straws, a more adequate correlation was proposed by Hata el al. (13), which takes into account both the diameter (D) and the length (L) of the straw. This correlation predicts h values that are 1.52 to 2.77 times higher than the h values for vertical plates. The experimental value of h=16.7 W/m^2°C obtained in the present work is within the range of values predicted by Hata correlation (9-18W/m^2°C)

CONCLUSION

In this study, the surface heat transfer coefficients for plastic French semen straws placed in nitrogen vapor over liquid nitrogen were determined from the measurement of time-temperature curves during freezing, using a numerical finite element program that solves the heat transfer partial differential equation under transient state with convective boundary conditions. The computational program was applied to simulate the freezing process of semen filled straws and an excellent agreement between experimental and calculated time-temperature values was observed for vertically and horizontally placed straws in nitrogen vapors.

The h values experimentally obtained were further compared to those calculated using correlations based on the Nusselt number for natural convection. The h value for horizontally placed straws obtained was lower than the value obtained by Nusselt correlations considering a natural convection situation: this can be attributed to the stratification of the nitrogen vapors. For vertical positioned straws there is a non-uniform ice fraction distribution in the axial direction due to the different external nitrogen vapor temperatures to which the straw is exposed. The numerical simulations validated against experimental measurements, combined with accurate h values provide a reliable tool for the prediction of freezing curves of semen-filled straws immersed in nitrogen vapor at different temperatures. The present study contributes to the understanding of the cryopreservation techniques for sperm freezing using engineering techniques, improving the cooling protocols and the manipulation of the straws.

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