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Abstract: Slush nitrogen (SN2) is a mixture of solid nitrogen and liquid nitrogen coexisting under an average temperature of -207ºC. In order to investigate whether plunging a French plastic straw (commonly used for spermatozoa cryopreservation) in SN2 may substantially increase cooling rates with respect to liquid nitrogen (LN2), a numerical simulation of the heat conduction equation with convective boundary condition was used to predict the cooling rates. Calculations performed using heat transfer coefficients in the range of film boiling confirmed the main benefit of plunging a straw in slush over LN2 does not arise from their temperature difference (-207ºC vs. -196ºC) but from an increase in the external heat transfer coefficient. Numerical simulations using high heat transfer (h) coefficients (assumed to prevail in SN2) suggested that plunging in SN2 would increase cooling rates of French straw. This increase of cooling rates was attributed to a less or null film boiling responsible for the low heat transfer coefficients in liquid nitrogen when the straw is placed in the solid-liquid mixture or slush. In addition, it was demonstrated that predicted cooling rates of French straws in SN2 tends to level-off for high h values, suggesting heat transfer is dictated by the heat conduction within the liquid filled plastic straw.

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1. Introduction

The plastic straw was first introduced in Denmark by Sorensen [1] in 1940 for packing liquid semen and the first attempts of freezing straws in liquid nitrogen (LN_2) were reported by Adler [2] in 1961 and later modified by Cassou [3] and Jondet [4]. A typical commercially available straw is made of polypropylene or polyvynil chloride having approximately 130 mm length, 2.6 mm o.d , 1.9 mm i.d , with 0.35 mm plastic wall thickness.

Pool boiling occurs when a relatively large volume of fluid surrounds the surface of a submerged object, and the fluid is not flowing itself. As soon an object is plunged into LN_2 it enters into the so-called film boiling regime due to the large temperature difference between the object and LN_2 [5, 6]. This determines a heat flow from the object to LN_2 causing the latter to boil in the immediate vicinity of the object creating a pocket of nitrogen vapor around it which acts as "insulator" and retards further heat transfer. Film boiling is also referred to as the "Leidenfrost effect" [7]. The object then will cool down, rather slowly due to the low heat transfer rates and the "minimum heat flux" point will be reached. Vapor film will then break off while the heat flux progressively increases as transition boiling regime is established. It is only at this point that nucleate boiling is reached; this event is characterized by a steep increase of the heat flux and up to a point called the" maximum heat flux" [5, 8].

Current methods of sperm cryopreservation contained in a plastic French straw involve equilibration and subsequent plunging into LN_2, which results in strong nitrogen vaporization around its surface, creating a "vapor coat" which surrounds the straw acting as a heat-insulation layer or film boiling regime. As a result, convective heat transfer coefficient (h) at the interface between the straw and the liquid nitrogen is quite limited leading to low cooling rates [9, 10].

High freezing rates have been associated with higher cell survival by several authors [11]. When a pre-cooled plastic straw (i.e., 6ºC) is immersed in LN_2 having a boiling temperature of -196ºC, the liquid nitrogen in contact with the straw surface immediately enters into the film boiling regime due to the large temperature difference between the straw and LN_2 (ΔT=190ºC). According to Chao [12] film boiling regime is observed at $\Delta T > 30$ K. Sansinena et al. [13] theoretically predicted the effect of heat transfer parameters on cooling rates of liquid filled plastic French straw and concluded that in order to obtain high cooling rates, conditions had to be designed to reach the highest possible heat transfer coefficients when the plastic straw is plunged in LN_2.

In 2002, Arav [9] developed a system where the temperature of liquid nitrogen was reduced by applying negative pressure, obtaining a mixture of solid and liquid nitrogen. The resulting slush nitrogen (SN_2) is a mixture of solid nitrogen + liquid nitrogen having a temperature of (average) -207 ºC. In slush nitrogen, the cooling rate attained when plunging a vitrification device (i.e. OPS "Open Pulled Straw", Cryotop) was dramatically increased as compared to LN_2. For this reason, SN_2 has been suggested in recent years as a way to improve cooling rates and survival of oocytes and embryos in vitrification procedures [10, 14, 15, 16]. Dos Santos et al. [17] determined the effect of SN_2 on the development of vitrified immature and mature bovine oocytes loaded in OPS. Nowshari and Brem [18] in their study with mouse embryos plunged 0.25 ml straws in SN2 (Vit-Master®chamber).

Liquid nitrogen has a temperature of -196ºC while SN_2 has a temperature in the range of -205ºC to -210ºC (average -207ºC). It was hypothesized that the main benefit of quenching with the slush instead of liquid nitrogen is not derived from the temperature difference between the two systems, but mainly from the fact that SN_2 minimizes the insulating vapor layer associated with LN_2 cooling. It is noteworthy that Katkov et al. [19] suggested that calculation of cooling rates was a convenient procedure due to difficulties associated with the direct measurement of temperatures in small cryopreservation devices. The aim of the present work is to perform a numerical simulation of cooling rates in plastic French straw (commonly used for spermatozoa cryopreservation) in order to verify if the utilization of SN_2 actually improves cooling rates. For this purpose, the unsteady-state heat conduction equation for concentric cylinders was numerically solved taken into account convective heat transfer coefficients which typically describe the straw plunging in LN_2 and SN_2 and assuming that ice formation during cooling was avoided (i.e. vitrification phenomenon).

2. Materials and methods

A commercially available polypropylene straw (130 mm length, 2.6 mm o.d, 1.9 mm i.d. and 0.35 mm wall thickness) was considered as the model system for the heat conduction. For modelling purposes two concentric cylinders were considered; the inner cylinder was assumed to be water-filled and the outer cylinder was the straw manufacturing material. Heat transfer through the straw wall and into the liquid column inside was considered to proceed by conduction. The physical model system was described in a previous publication [13].

The heat conduction partial differential equations for the axisymmetric problem in cylindrical coordinates, for each material i, (i=1 cryopreserved spermatozoa suspension; i=2 plastic straw material) is represented as follows:

$$\rho_i \, Cp_i \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_i \, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_i \, r \frac{\partial T}{\partial z}\right) \quad \text{in } \Omega i \qquad t>0$$

(1)

where Ωi 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, t is the cooling time , r and z are the radial and axial coordinates .

The continuity flux border condition at the interphase between the two materials and the symmetry condition at r=0 were considered. The boundary convective condition was expressed as:

$$-k_2\left(\nabla T \cdot n_2\right) = h \cdot (T - T_{ext})$$

(2)

where h is the surface heat transfer coefficient and T_{ext} is the external temperature of the liquid nitrogen. Constant thermal properties were considered. Besides the model was also solved considering a boundary condition of constant temperature at the interface that corresponds to the case of $h \rightarrow \infty$ in order to carry out a complete analysis of the effect of h on cooling rates.

The heat conduction equation with convective boundary condition (strong formulation) can be considered a linear mathematical problem and was solved using the finite element method (FEM) applying the variational formulation (weak formulation). A commercial FEM package (COMSOL Multiphysics) was used to simulate the cooling process of the systems. To facilitate estimation of cooling rates, it was assumed that vitrification avoided ice formation during cooling; i.e. no latent heat of ice crystallization was released. Thermal properties (thermal conductivity, specific heat and density) of all materials were those previously reported by [13]. The cooling rate (ºC/min) was defined as the time needed to reduce initial core temperature of the liquid column from 6ºC to -150ºC at the warmest point in the device.

3. Results and discussion

Heat transfer coefficients for plastic straws immersed in LN_2 have not been reported in the literature. Recently, Sansinena et al. [20] reviewed values of heat transfer coefficients for pool boiling of various small metallic objects (i.e., thin metallic wires, flat disks, etc) plunged in liquid nitrogen, and concluded they are in the range of 125 to 1000 W/(m^2 K) corresponding to film boiling regime. Previous studies [21] assumed that h for cryopreservation devices plunged in liquid nitrogen should be below 1000 W/(m^2 K), and this assumption is in agreement with the abovementioned values for film boiling in liquid nitrogen. Heat transfer coefficients for devices plunged in slush nitrogen are not available in literature; however it may be assumed they are well above the higher limit corresponding to film boiling 1000 W/(m^2 K).

Figure 1 shows predicted cooling rates for French straw when cooled either at -196ºC (LN_2) or -207ºC (SN_2), for various heat transfer coefficients estimated to be representative of film boiling (h ≤ 800 W/(m^2 K). It is observed that lowering the cooling temperature from -196ºC to -207ºC produces a slight increase (12 %) in cooling rates at any of the external heat transfer coefficients considered. When the model was run considering constant temperature at the surface ($h \rightarrow \infty$) the calculated cooling rates were 1376ºC/min and 1510ºC/min for -196ºC and -207 ºC respectively (an increase of 9.7%). Thus, main benefit of quenching with the slush instead of liquid nitrogen is not derived from the temperature difference between the two systems.

Figure 2 compares predicted cooling rates for French straw corresponding to LN_2 conditions (-196°C, h ≤ 800 W/(m^2 K)) with conditions supposed to prevail in SN_2 (-207ºC and h ≥ 2000 W/(m^2 K). As mentioned above, the actual heat transfer coefficient for a plastic straw plunged in LN_2 is not known although it is reasonable to suppose that lies within the film boiling regime values. Wesley-Smith et al. [21] suggested that slush nitrogen delayed the onset of film formation around the sample (Leidenfrost phenomenon) and Yoon et al. [22] reported photographs showing that no bubble formation occurred when a gold grid (containing oocytes) was immersed into slush nitrogen. Echlin [23] suggested that heat transfer improvement in slush nitrogen is because there is less film boiling when a sample is plunged in slush, since the heat is first transferred to the solid N_2 causing it to melt instead of producing the vaporization in N_2 film boiling regime.

It is important to highlight that predicted cooling rates in SN_2 (Figure 2, upper curve) tends to level-off for h values above 4000 W/(m^2 K). Besides, when the model for SN_2 was solved considering a boundary condition of constant temperature at the interface (-207ºC), that corresponds to the case of $h \to \infty$, the cooling rate for straw plunged in SN_2 became 1510ºC/min (value not shown in Figure 2). This cooling rate represents the case when heat transfer is solely controlled by internal conduction and may be compared with the calculated rates for high heat transfer coefficients. The value of 1510ºC/min (internal control only) is only 6.6 % and 9.9 % higher than cooling rates corresponding to h values of 6000 and 4000 W/(m^2 K), respectively. From the results it can be concluded that heat transfer becomes controlled by heat conduction within the liquid filled straw and the plastic material.

To analyze the improvement of SN_2 (-207ºC) performance with respect to LN_2 (-196ºC) the cooling rates were compared for two specific conditions. The first example corresponds to h = 800 W/m^2 K for LN_2 that produced a cooling rate 936ºC/min compared to SN2 with a h = 6000 W/(m^2 K) that led to 1418.2 ºC/min; this resulted in a 51.5 % of increase in the cooling rate. The second analysis was done considering h= 400 W/(m^2 K) for LN_2 which led to a cooling rate of 682ºC/min, compared with SN_2, considering h = 4000 W/(m^2 K); in this case a two-fold increase in cooling rate was achieved. It is noteworthy that for minimal volume systems (i.e., 1 µl), immersion in slush nitrogen was observed to have a profound effect on cooling rate due to an important role of external control in the heat transfer phenomenon [24].

From these results, it can be concluded that the SN_2 cooling velocities would be significantly higher than the LN_2. However, the performance of SN_2 in the case of French straw (i.e. cooling rates) is limited by the geometry and large dimensions of the system requiring the use of cryoprotectants that suppress the formation of ice crystals to attain vitrification [25].

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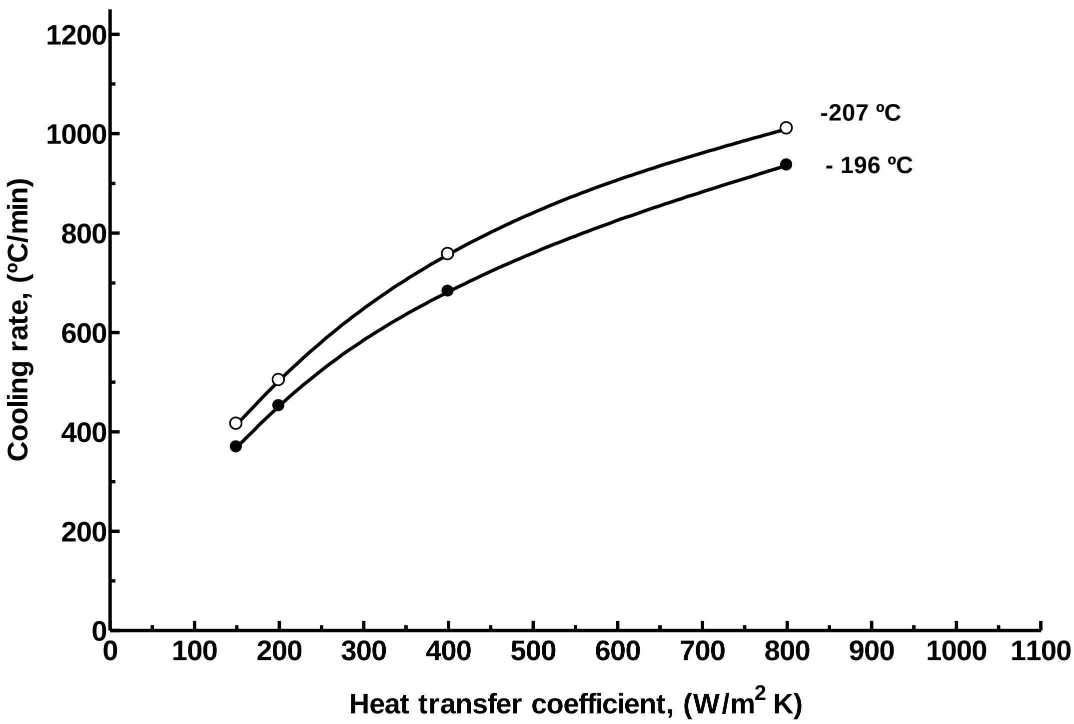

Figure 1. Comparison of calculated cooling rates for French straw at -196ºC and -207ºC in a range of heat transfer coefficients estimated to be representative of film boiling.

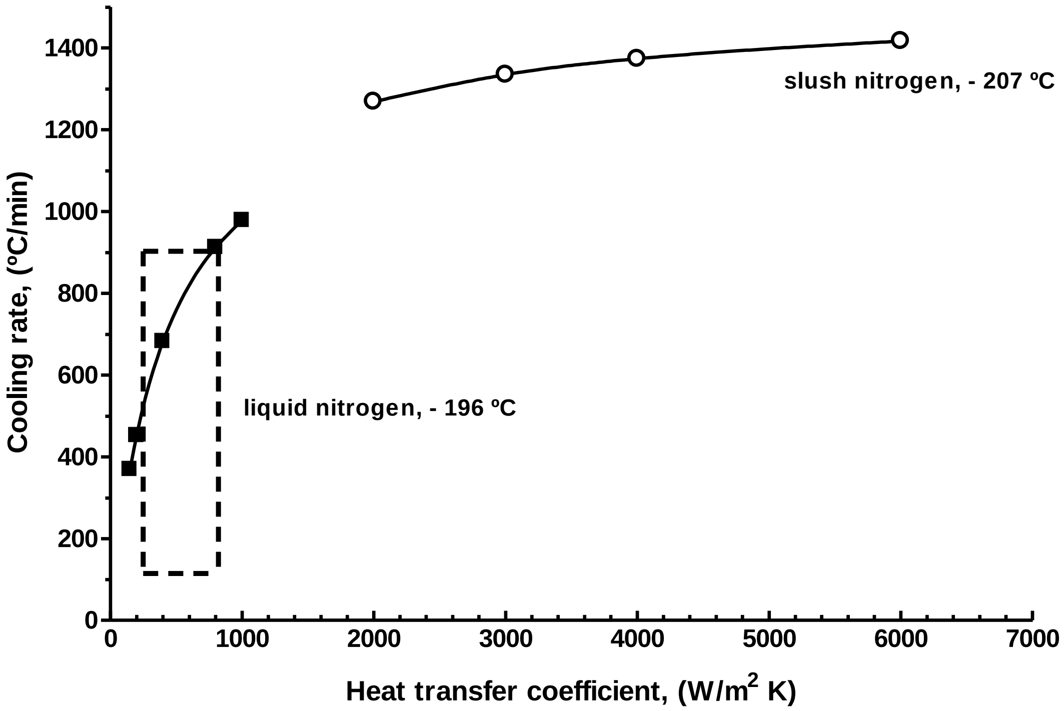

Figure 2. Comparison of calculated cooling rates for French straw corresponding to liquid nitrogen conditions (-196°C, h ≤ 800) with conditions assumed for slush nitrogen (-207ºC, h ≥ 2000). The rectangle indicates probable range of h values for film boiling regime.