

Cryopreservation of Human Sperm: Effect of Cooling Rate on Intracellular Ice Formation

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Abstract-The effect of cooling rate on the intracellular ice formation for human spermatozoa have been studied and a correlation formula is established in this study. It is found that after a certain cooling rate, termed as optimal cooling rate in this study, the volume of intracellular ice increases exponentially with increase in the cooling rate. The cellular response of human sperm cells to different cooling rates, varying between 5°C/min and 300°C/min, is studied using the diffusion limited transport model. The membrane permeability parameters are taken from the published literature. For a cooling rate lower than the optimal value, almost all intracellular water flows out to the extracellular compartment through the cell membrane leaving behind only the osmotically inactive cell volume inside the cell. For the calculation of the amount of intracellular ice formation, it has been assumed that the amount of water trapped inside the human sperm at -19°C will finally form intracellular ice with sufficient super-cooling.

Index Terms: Cryopreservation, Human sperm, Numerical modeling, Optimal cooling rate, Correlation, Intracellular ice formation, Diffusion limited water transport.

1 INTRODUCTION

History suggests that sperm cryopreservation had started in the late 1950's and in the last few decades there have been enormous developments in the field of its preservation at low temperatures [1],[2],[3]. It became a key area of scientific investigation when the dairy industry needed long term storage methods for preservation of bull sperm[4]. This area of research came into prominence only when the foundation of human sperm cryopreservation was laid by Polge et al in 1949 discovering the use of glycerol as a cryoprotective agent and that it could provide protection to the cells at low temperature [5]. In the early nineteenth century Montegazza, discovered that human sperm can actually survive at temperatures below -15°C but the majority of these sperms could not survive the damaging effect of ice formation (i.e. freezing injury). The supplementary deciding factors for thriving cryopreservation are the sperm dilution rate, cooling rate, thawing rate and the

rate of intracellular ice formation, which is the main cause of cellular damage. The ice formation is a significant and detrimental factor which affects cryopreservation. Better understanding of intercellular ice formation rate will eventually lead to the production of many viable sperm cells. Also, apart from the change in the rate of intercellular ice formation a successful sperm cryopreservation requires maintaining the post-thaw structural and functional integrity [6],[7],[8],[9],[10],[11].

Though there has been incessant progress in the field of sperm cryopreservation but still there are many questions to be answered and many baffling puzzles to be solved. Hence, the main objective of this study is to adjoin one more footstep for the proper preservation of human sperm by simulating the optimal cooling rate.

And this will quantify the effect of cooling rate on intracellular ice formation for human spermatozoa cell lines. The biophysical properties of human sperm are taken from the published literature [7].

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2 GOVERNING EQUATIONS

The water transport during cryopreservation of a cell is driven by a chemical potential difference between the intracellular and extracellular solution. The intracellular water moves towards the extracellular solution which leads to the volumetric shrinkage of the cell as a result of this potential difference. Mazur [8] proposed a theoretical model for water transport across the cell membrane which was later modified by Levin et al. [9] and is given as,

$$\frac{dV}{dT} = -\frac{L_p A_c R T}{B v_w} \left[\ln \frac{(V_0 - V_b)}{(V_0 - V_b + \phi_s n_s v_w)} - \frac{\Delta H_f v_w \rho}{R} \left(\frac{1}{T_R} - \frac{1}{T} \right) \right] \quad (1)$$

Where L_p is the sperm membrane permeability to water, given as below

$$L_p = L_{pg} \exp \left[-\frac{E_{Lp}}{R} \left(\frac{1}{T} - \frac{1}{T_R} \right) \right] \quad (2)$$

L_p is an Arrhenius function of L_{pg} and E_{Lp} .

2.1 Code Validation

The present numerical code is validated against the published experimental result of Devireddy et al. [7]. In the experiment, the volumetric response of human sperm as a function of subzero temperatures was obtained using the differential scanning calorimeter technique. Figure 1 shows a comparison between numerically computed volumetric response of human sperm and the experimental measurements.

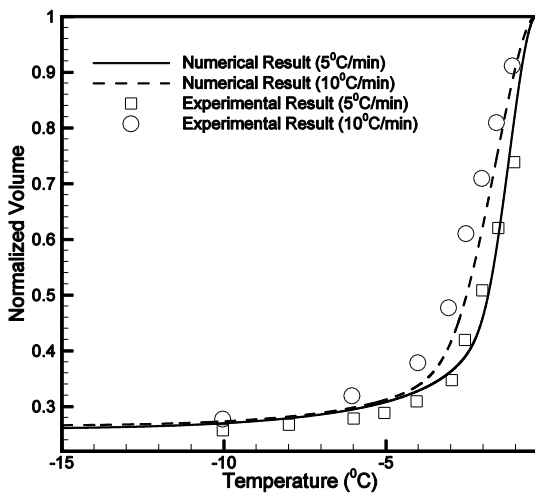


Fig1. Validation of the present numerical code.

The rectangles and circles represent experimental values for a cooling rate of 5°C/min and 10°C/min respectively and the solid line and dotted line represent the numerical result for the cooling rates of 5°C/min and 10°C/min respectively. The numerical results match quite well with the experimental results validating our numerical model.

TABLE 1

BIOPHYSICAL PROPERTIES FOR HUMAN SPERM FROM DEVIREDDY ET AL. [7].

Symbol	Value	Unit
T_R	273.15	K
r_0	0.42	μm
L	40.2	μm
A_c	106	μm^2
V_0	22.2	μm^3
V_b	0.23 V_0	
R	8.314	J/mol K
v_w	18×10^{12}	$\mu\text{m}^3/\text{mol}$
ΔH_f	335	mJ/mg

3 RESULT & DISCUSSION

In this study, the human sperm is modeled as a long cylinder with biophysical properties given in Table 1. The osmotically inactive cell volume (V_b) is assumed to be 0.23 V_0 . The values of two membrane permeability parameters are taken as the best fit parameters obtained from the published results. To assess the effect of cooling rate on intracellular ice formation the numerical results are obtained for different cooling rates varying from 5°C/min to 300°C/min. It has been assumed that the amount of water trapped inside the human sperm at subzero temperature of -19°C will finally form the intracellular ice with sufficient super-cooling. (In the figure, the cooling rate is increasing from 5°C/min to 300°C/min as we proceed from the bottom to top)

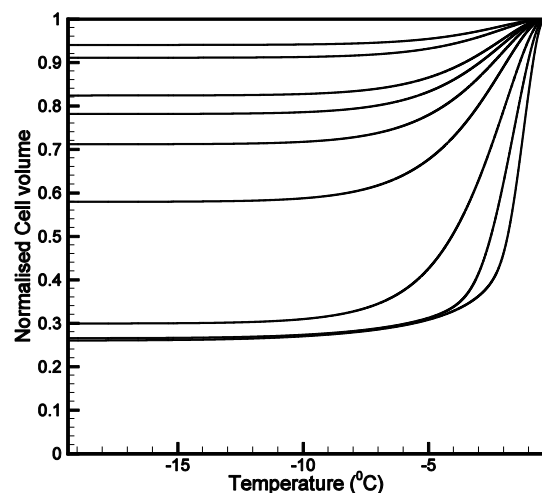


Figure 2. Normalized cell volume at different cooling rates varying from 5°C/min to 300°C/min.

Figure 2 shows the volumetric response of human sperm for cooling rates of 5°C/min, 10°C/min, 20°C/min, 40°C/min, 60°C/min, 80°C/min, 100°C/min, 200°C/min, 300°C/min (from bottom to top). As the cooling rate increases the amount of water trapped inside the cell increases till the higher cooling rate is reached, after which there is a constancy in the volume of intracellular water. It should be noted that up to a certain cooling rate (termed as optimal

cooling rate in this paper) all the water inside the cell leaves the intracellular compartment leaving behind the osmotically inactive cell volume as the end volume. The value of the optimal cooling rate is found to be nearly equal to 17°C/min. After the optimal value the trapped water volume increases with increase in the cooling rates as reflected in the figure. Also, this increase is not linear with the increase in cooling rate.

TABLE 2 NOMENCLATURE

V_0 Isotonic sperm volume	v_w Molar volume of water
V_b Osmotically inactive sperm volume	ΔH_f Latent heat of fusion of water
L_{pg} Reference membrane permeability	
E_{LP} Apparent activation energy	Greek Symbols
A_c Effective membrane surface area	ϕ Disassociation constant
	ρ Density of water, m3
L Sperm length, mm	Subscripts
R Universal gas constant (J/molK)	s salt
T_R Reference temperature (K)	w water
B Cooling rate (K/min)	

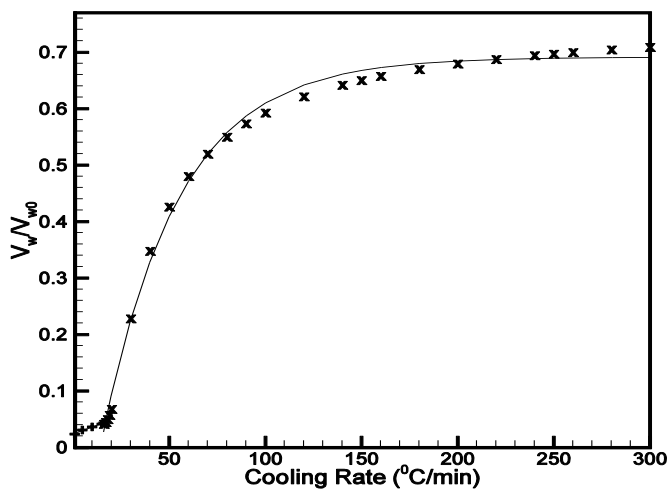


Fig 3. Correlation between the cooling rate and the volume of intracellular ice formation.

Figure 3 shows the correlation when the cooling rate and the normalized trapped water. The plot is showing a curve which increases linearly till the optimal cooling rate and thereafter it increasing exponentially with the increase in cooling rate. This indicates that, after a certain range of cooling rate called as optimal cooling rate the volume of trapped water is increasing with increase in cooling rate by forming more amount of intracellular ice. If the cooling rate is much higher than the optimal value then water inside the cell will not get sufficient time to exit and will form ice within the cellular environment which may either lead to crystal formation or rupture of the cell wall and other cellular injuries.

With the numerical data obtained for intracellular ice volume, a correlation formula is established between the cooling rate and the volume of intracellular ice formation to justify the effect of cooling rate on intracellular ice formation for a human sperm. The correlation is described by the following equation:

$$\frac{V_w}{V_{w0}} = a + be^{-(c.CR)} \quad (3)$$

Where $a=0.6904$, $b= -0.9916$, $c= 0.0251 \text{ min}/^\circ\text{C}$ and V_w = volume of water trapped, V_{w0} = initial water volume, CR = cooling rate.

4 CONCLUSION

A correlation formula between the cooling rate and the volume of intracellular ice formation was obtained for human sperm in this study. The intracellular ice formation during different cooling rates was studied within a range from $5^\circ\text{C}/\text{min}$ to $300^\circ\text{C}/\text{min}$. It was found that after a certain cooling rate called as optimal cooling rate (which is $\sim 17^\circ\text{C}/\text{min}$ in this case) the intracellular ice formation increases exponentially. The correlation established in the current study will help in finding the optimal cooling rate for other mammalian sperm cells as they exhibit almost same biophysical properties during freezing and this will enhance our knowledge for optimizing equine sperm cryopreservation along with other mammalian sperm cells.

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