REAL TIME DETERMINATION OF CELL VOLUME CHANGES OF SPERM USED FOR CRYOPRESERVATION

INTRODUCTION

Much of the cellular damage produced by freezing is due to freezing-induced dehydration and thawing-induced rehydration. Both dehydration and rehydration impose anisotropic mechanical stresses on membranes and macromolecules. The measurement of change in cell volume may be correlated with cellular freezing and thawing damage. Study of cell volume osmotic induced changes may help improving cell cryopreservation processes.

- **Freezing and thawing of cell suspensions**

Cooling rates are "slow" and "fast" depending on whether heat leaves the cell more slowly or more quickly than water does. The extracellular solution almost always freezes first. The extracellular solutes are concentrated in the remaining unfrozen extracellular water, and, if cooling rates are slow enough, this dehydrates the cell by osmosis as water diffuses from the cytoplasm into the more concentrated external solution. The characteristic time for water to equilibrate across the plasma membrane is only ~ tenths of a second.

Artificial cryopreservation usually uses high and very high cooling rates. Consequently, there is insufficient time for much water to leave the cell, so supercooling occurs with relatively little osmotic contraction. Supercooling can lead to intracellular ice formation, which is fatal. If the cooling is sufficiently fast, and if the intracellular solution is sufficiently concentrated and viscous, vitrification of the cytoplasm can occur. Vitrification means formation of a glass, meaning an extremely viscous solution which is not in thermodynamic equilibrium, but which remains away from equilibrium for a very long time (ice cream is a familiar example of such a substance). The total internal solute concentration of cells and organisms is rarely high enough to allow vitrification. It can be increased in two ways - by osmotic contraction (i.e. using the cell's own solutes) or by adding to the suspending medium some solutes that are able to penetrate cell membranes. In an aqueous glass, water molecules are not in their stable state, but the high viscosity prevents them from moving around and "finding" their stable, crystalline state. Paradoxically, freezing can occur in a glass when it is warmed. As the temperature rises (but while still below the equilibrium freezing temperature), the viscosity of the glass falls and its molecular motion increases. This allows the molecules to move and to rotate---and sometimes to form ice crystals.

- **Osmotic Induced Volume Changes in Spermatozoa**

Since low temperature influences phase transitions, it is likely that temperature can moderate the osmotic tolerance of sperm and may result in a lower

tolerance range below phase transition temperatures.

An indirect estimation of cell volume may be obtained by measuring cell permeability. Permeability in macaque sperm at subzero temperatures may be measured using the Differential Scanning Calorimetry (DSC) technique. This technique has been used to define the optimal cooling rate for spermatozoa The DSC continuously measures heat releases associated with phase change as a function of both time and temperature at controlled cooling rates. The technique is based on measuring the difference in integrated heat release between an initial freezing of intact cells and a final heat release from freezing of the lysed cells. This value has been shown to be proportional to the volume of osmotically active cell water in the sample prior to freezing. In addition, the temperature dependence of the integrated heat release difference normalized by the total heat has been shown to be proportional to the normalized amount of cell water that has left the cells. This allows sperm cell volume to be calculated using the DSC measured heat releases. The relationship of the cell volume with respect to temperature can be calculated using a mathematical formula.

A direct, quick and easy measurement of cell volume is possible using the Multisizer 3. Cell volume changes are easily quantified during an osmotic challenge of a cell suspension. The pulse data generated during the analysis shows the cell volume at any time within the analysis.

MATERIALS AND METHODS

A Beckman Coulter® Mulitisizer™ 3 COULTER COUNTER® was used to measure the sperm cells. A 100µm aperture tube was used and the analyses were run for 20 seconds.

A cell suspension was prepared in a round bottom beaker. An analysis was run to determine the stability of cell volume.

A known volume of water was added to the cell suspension and the analysis was run again to determine how a change in media osmolalilty affected the cell volume.

RESULTS

Figure 1 shows the pulse graph for the control; cell volume is stable throughout the analysis.

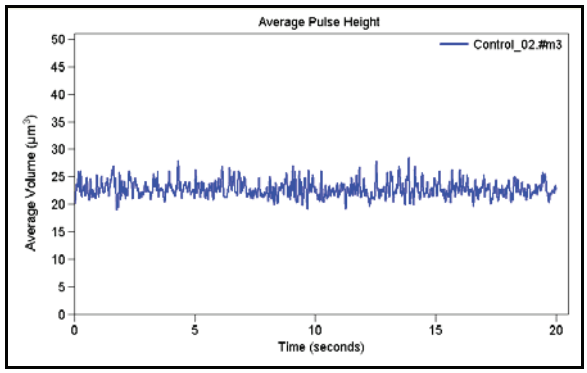

Fig. 1

When water was added to the suspension, the effect in cell volume due to the change in osmolality of the media is shown in Figure 2.

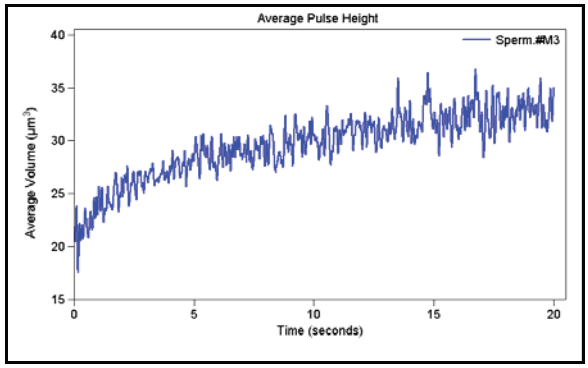

Fig. 2

The cell volume at any time during the analysis may be known by clicking on the graph (Fig. 3). The Multisizer 3 software displays a detailed list of cell volume against time.

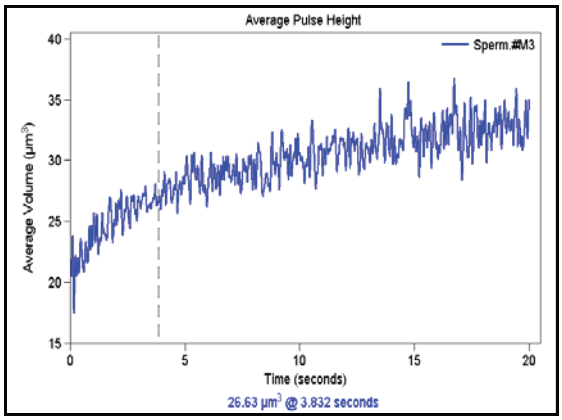

Fig. 3

In addition to the mean cell volume of sperm at different times, we were also able to obtain the size distribution of the cell population at different time intervals. Figures 4 and 5 show the size distribution for 3 to 4 seconds and 15 to 20 seconds intervals.

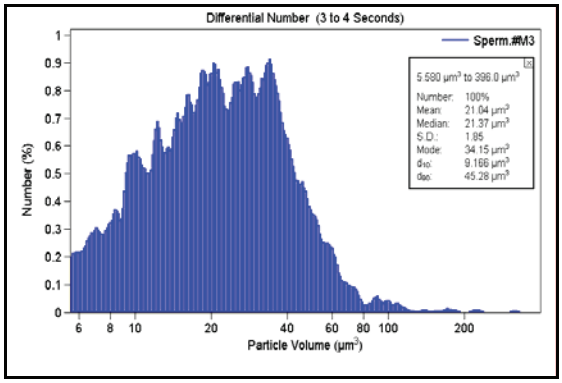

Fig. 4

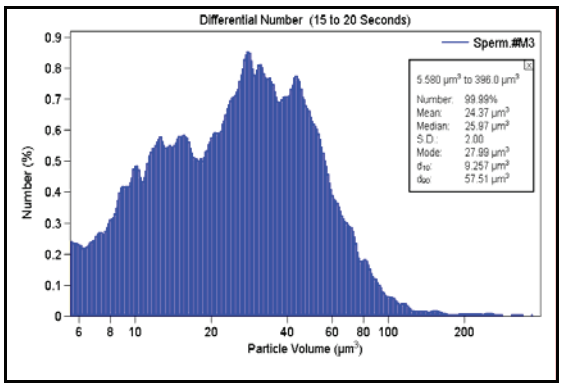

Fig. 5

Table 1 shows the mean cell volume every two seconds during the 20 second analysis.

Size Distribution	MCV (μm^3)	Elapsed Time (sec.)
SP 1	22.24	2
SP 2	25.09	4
SP 3	26.77	6
SP 4	27.54	8
SP 5	28.27	10
SP 6	29.08	12
SP 7	29.68	14
SP 8	30.28	16
SP 9	30.66	18
SP 10	31.06	20

Table 1 Mean Cell Volume (MCV) and different analysis times

CONCLUSIONS

The direct measurement of change in cell volume is a fast, simple and accurate analysis using the digital pulse processor of the Beckman Coulter Multisizer 3.

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