Motility Comparison of Epididymal Rat Sperm after Exposure to Various Molar Concentrations of Trehalose

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Summary

In this study, rat epididymal spermatozoa were collected and subjected to hyperosmotic solution induced by various molar concentration of trehalose in two different base solution namely, HEPES buffered Tyrode's lactate (TL-HEPES) and modified Krebs Ringer solution to determine the potential use of a new cryoprotective agent for the cryopreservation process. The two media, mKRB and TL-Hepes, consisted of 20% egg yolk and .5% Equex Paste. The sugar trehalose was added to create aliquots of increasing molar concentration beginning with isosmotic (no trehalose, 275 ± 5 mOsm) plus 0.1, 0.2, 0.3, 0.4 or 0.5M trehalose. The sperm samples collected from cauda epididymides of sexually mature (12-15 weeks) Sprague Dawley rats were exposed to these various concentrations of trehalose solutions, held for 5 min and then returned to isosmotic mKRB or TL-Hepes solution. We evaluated motility characteristics such as total motility, progressive motility, and average path velocity. Flow cytometry was also used to assess acrosome integrity of the sperm. We expect that the results will reveal an optimal sperm freezing extender for epididymal rat sperm.

Background

The rat is considered to be one of the most valuable laboratory animals with regards to research in modern medicine and physiology. With the constantly expanding number of mutant and transgenic rat strains, the importance and difficulty of cryopreserving rat spermatozoa is widely recognized among the scientific community. Total motility, progressive motility and average path velocity are commonly used to assess the quality of a sperm sample, and accordingly, we used these measurements in this study. Acrosomal integrity can be easily measured by using fluorescein staining followed by flow cytometry and is another indicative quality currently used to determine the health of sperm samples.

Materials and Methods

Computer Assisted Semen Analysis: Hamilton Thorne Computer-assisted sperm motility analysis system was used to analyze rat sperm motility characteristics (motility, progressive motility and average path velocity). Sperm samples were put onto a pre-warmed 80 µm deep dual sided chamber (2X CELL, Hamilton Thorne Inc). Sperm motility was determined at 37°C. At least 6 fields were counted and the measurements were replicated 2 times for each donor.

Evaluation of Acrosomal Integrity: Acrosomes were analyzed using fluorescein staining and flow cytometry. Samples were prepared as for motility then incubated with 20 µg/ml Alexa Fluor-488-PNA at 37°C . Stained sperm samples were then counted (+-10,000 cells/sample) using flow cytometry. Microscopic images of fluorescein-stained sperm are given in Fig. 1.

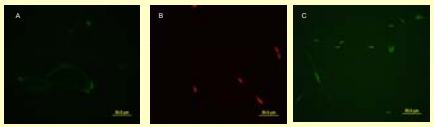

Figure 1: The representative fluorescent images of rat sperm after staining with Alexa Fluor-488-PNA, PI or SYBR-14. (A) Solid line indicates intact acrosome and broken line shows damaged or lost acrosome; (B) membrane damaged and (C) membrane intact rat sperm.

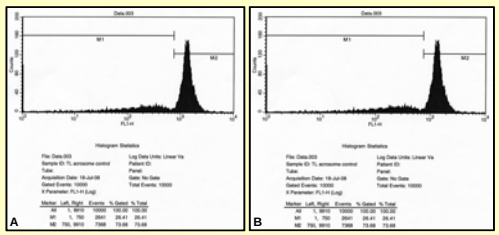

Figure 2a and 2b. Examples of flow cytometry. Figure 2a illustrates a fluorescein stained control for TL-Hepes solution with 73.7% intact acrosomes. 2b shows an example of a trehalose treatment in the egg yolk solution. In this case, the sample solution contained 48.2% intact acrosomes.

Results

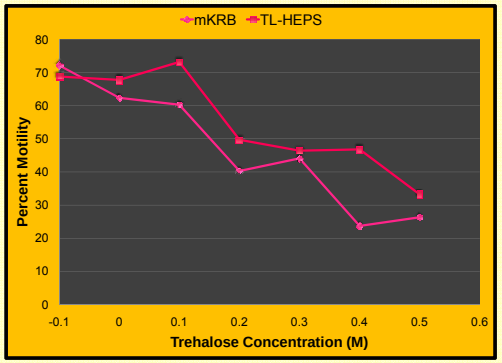

Figure 3: Percent motility (means +- 5.12 SEM, P<0.05) of epididymal rat sperm treated with increasing concentrations of trehalose in egg yolk solution. (-0.1 indicates the control)

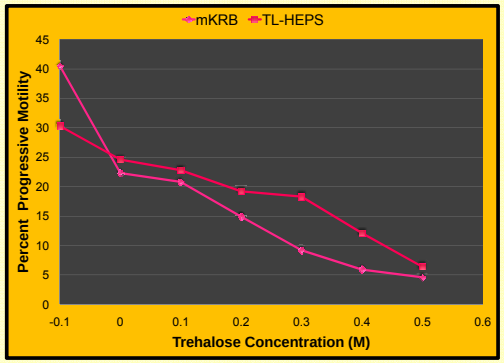

Figure 4: Percent progressive motility (means +- 3.53 SEM, P<0.05) of epididymal rat sperm treated with increasing concentrations of trehalose in egg yolk solution. (-0.1 indicates the control)

Trehalose Concentration (M)	TL-Hepes	mKRB
Control	73.7	63.5
0	52.1	47
0.1	42.9	50
0.2	51.9	44.7
0.3	57.8	47.5
0.4	58.6	54.6
0.5	46.8	48.2

Figure 5: Percent intact acrosomes of rat sperm treated with increasing concentrations of trehalose .

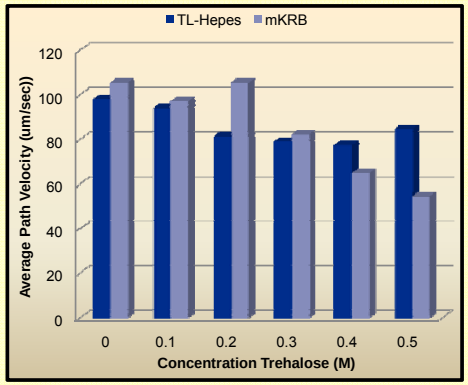

Figure 6: Average Path Velocities (um/sec) of epididymal rat sperm treated
Increasing concentrations of trehalose.

Conclusions

This study showed that rat sperm is extremely sensitive to increasing concentrations of trehalose. Even concentrations as low as 0.2M showed noticeable differences. It is also indicated that acrosome integrity is not affected by varying concentrations of trehalose. TL-Hepes and mKRB egg yolk solutions displayed similar patterns in regards to motility and acrosome integrity, and neither solution appears to be preferable to the other.

Acknowledgements

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