MOTILITY, VIABILITY AND APOPTOSIS IN CRYOPRESERVED HUMAN SPERM WHEN THAWED AT EITHER ROOM TEMPERATURE OR 37°C

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INTRODUCTION

Sperm cryopreservation has many clinical uses, from the quarantine of donated semen to fertility preservation for men undergoing vasectomy or cancer treatment. The WHO in its 5th Edition manual (2010) documents protocols for sperm freezing using a cryoprotectant containing egg yolk and glycerol. Unfortunately, the use of egg yolk is frowned upon these days and so commercial cryoprotectants featuring glycerol in a pH buffer have become common. However, the WHO do describe the cooling of semen straws in nitrogen vapour (approximately -80°C to -100°C) as an acceptable method. Cooling by holding the straws at room temperature for 6 minutes can then be undertaken, although the effect of this extended exposure in the liquid phase to the potentially cytotoxic glycerol should not be underestimated. Nevertheless, sperm freezing/thawing is not standardized and so many laboratories use their own protocols that seem to work well, although it would seem reasonable to continually try to improve sperm survival by systematically studying the different components of such protocols.

Sperm survival after freeze/thaw procedures is often gauged by assessing motility, but there is increasing evidence that subtle damage not apparent under the light microscope is caused by freeze/thaw. Whilst oxidative stress inevitably plays a role in sperm cryo-injury (Thomson et al, 2009), apoptosis markers have been shown to increase in sperm following freezing and thawing (Said et al, 2010).

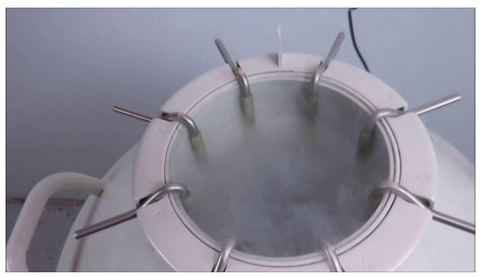
The present study used semen frozen with a glycerol-based cryoprotectant in nitrogen vapour. A thaw time of 3 minutes was set, and aliquots of each semen sample were thawed either at room temperature (RT) or in a water bath at 37°C. The post-thaw condition of the sperm was then assessed by quantifying sperm motility, sperm viability, and early and late apoptosis.

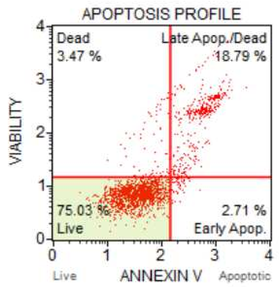
MATERIALS AND METHODS

Normozoospermic semen samples (n=7) were obtained from patients following informed consent. 0.5ml Quinn's AdvantageTM Sperm Freezing Medium was added dropwise to 0.5ml semen. Semen in two 0.25mL straws were placed vertically for 30 minutes into a goblet suspended in a Dewar, plunged into liquid nitrogen, and stored for up to 90 minutes before thawing. One straw was thawed at RT for 3 minutes whilst the other straw was thawed by placing it in a 37°C water bath for 3 minutes.

Sperm apoptosis was measured using Annexin-V flow cytometry as described by Drake-Brockman et al (2018). Samples for analysis of apoptosis were diluted to 0.5 million/mL using HTF. 100µL of Annexin-V and Dead Cell Reagent (Merck Millipore, Germany) was added to 100µL of the two aliquots of diluted sperm and incubated for 20 minutes in the dark according to the manufacturer's instructions. Annexin-V binding to phosphatidylserine was measured using a Muse Cell Analyzer flow cytometer (Merck Millipore, Germany).

Changes post-thaw were analysed by repeated measures ANOVA, and differences between thaws at RT and 37°C by paired t-test. Results with $p<0.05$ were considered significant.





POST-THAW ASSESSMENT OF SPERM

Overall post-thaw, there were:

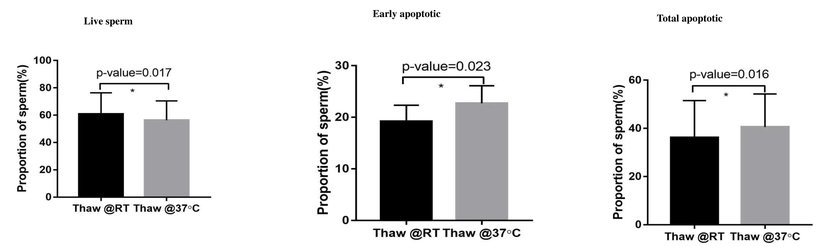
(a) significant reductions in total motility ($p<0.00001$), progressive motility ($p<0.001$), and the proportion of live sperm ($p<0.0001$)

(b) significant increases in dead sperm ($p<0.00001$), early ($p<0.00001$), late ($p<0.01$) and total apoptosis ($p<0.00005$).

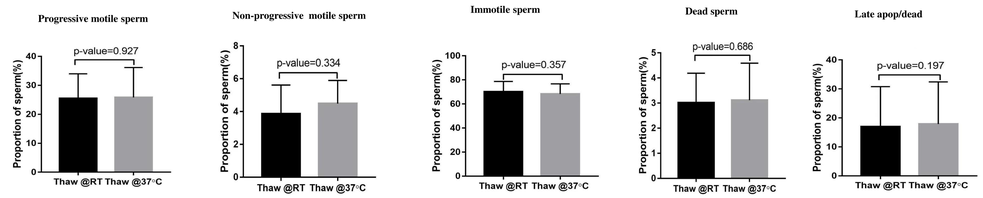
Compared to the RT thaw, the 37°C thaw resulted in:

(a) Significantly fewer live sperm,

(b) Higher incidences in early and total apoptosis.



(c) No difference in the proportion of sperm that are progressively motile, non-progressively motile, immotile, dead or showing signs of late apoptosis



SUMMARY

In summary, thawing frozen semen for 3 minutes at RT results in similar total and progressive motility compared to thawing at 37°C, but more live sperm and fewer showing early and total apoptosis. When thawing for 3 minutes, RT and not 37°C is therefore recommended.

REFERENCES

Drake-Brockman, L, Boisen B, Sanders S, Burton P, Effect of density gradient centrifugation and processing time on human sperm apoptosis. Journal of Reproduction Biotechnology and Fertility 2018; 7:38-46.

Said, T.M., A. Gaglani, and A. Agarwal, Implication of apoptosis in sperm cryoinjury. Reproductive biomedicine online 2010; 21(4): 456-462.

Thomson, L.K., et al., Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Hum Reprod 2009; 24(9): 2061-2070.

WHO, WHO laboratory manual for the examination and processing of human semen. 5th Edition ed. 2010, Geneva, Switzerland: World Health Organization.

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