Impacts of cooling and cryopreservation on human sperm capacitation, as measured by Cap-Score™

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Abstract

Introduction: Studies with fresh human sperm have shown that G_{M1} localization patterns (Cap-Score™) quantify capacitation status. Using an outcome of clinical pregnancy, Cap-Score prospectively predicted a man's fertility and determined his probability of generating a pregnancy. Here, we evaluate the impacts of cooling and cryopreservation/thawing on capacitation using Cap-Score.

Methods: Semen was collected, liquefied and split into control and experimental treatments. Control samples were processed normally for Cap-Score. For the cooling experiments, samples were extended in TEST Yolk Buffer (TYB) and cooled overnight in a Styrofoam box with a cold pack (n=5). For the cryopreservation experiments, samples were frozen in TYB with glycerol (Cryo; n=10). After storage in LN_2, the samples were thawed at 37°C for 3 min, mixed and then placed back into the water bath for another 3 min. Post-treatment, samples were washed, exposed to non-capacitating (NC) or capacitating (Cap) conditions, incubated for 3 hrs. and then Cap-Score was determined after an overnight fix.

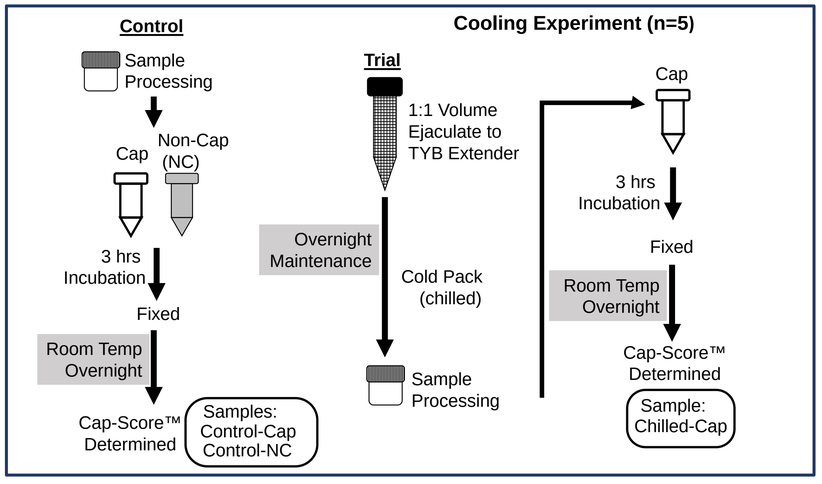
Results: An increase was observed in the control Cap when compared to the control NC treatment in the cooling experiment (40±4 vs 24±4%; p<0.01). There was no difference between the control Cap and the experimental Cap with cooled sperm (40±4 vs 40±2; p=0.87). In the cryopreservation experiment, an increase was again seen in the control Cap over the control NC (33±3 vs 19±2; p<0.01). Cap-Score was unchanged for Cryo Cap when compared to control CAP (34±1% vs 33±3%; p=0.75). No difference was observed between the Cryo NC and Cryo Cap (33±3 vs 34±1; p=0.82). The Cryo NC was greater than the control NC (33±3 vs 19±2%; p<0.01).

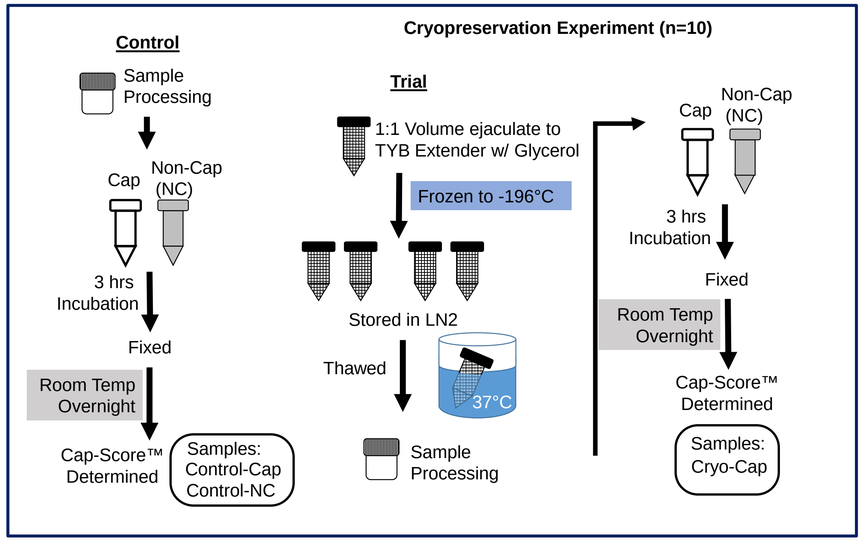
Conclusion: Despite exposure to TYB or TYB with glycerol, the Cap-Score male fertility assay could still be performed. Semen extension in TYB and overnight maintenance at reduced temperature had no detectable impact on Cap-Score. In contrast, cryopreservation/thawing in TYB with glycerol induced capacitation-like membrane changes in sperm incubated under non-capacitating conditions, supporting reports in the literature of the "cryocapacitation" phenomenon. However, no differences were observed in Cap-Score between fresh sperm or sperm after freezing/thawing and then incubation with stimuli for capacitation. Identification of impacts on capacitation could optimize protocols intended to preserve male fertility as well as improve IUI and IVF outcomes.

Introduction

Sperm must mature functionally in the process of capacitation to become able to fertilize. Capacitation depends on membrane lipid changes, and can be assessed by redistribution of the ganglioside G_{M1}, the basis of the Cap-Score™ male fertility assay. Cap-Score functionally assesses male fertility and was prospectively shown to predict pregnancy. Here, we determined the impact of cryopreservation/thawing and cooling using Cap-Score.

Experimental design





Results

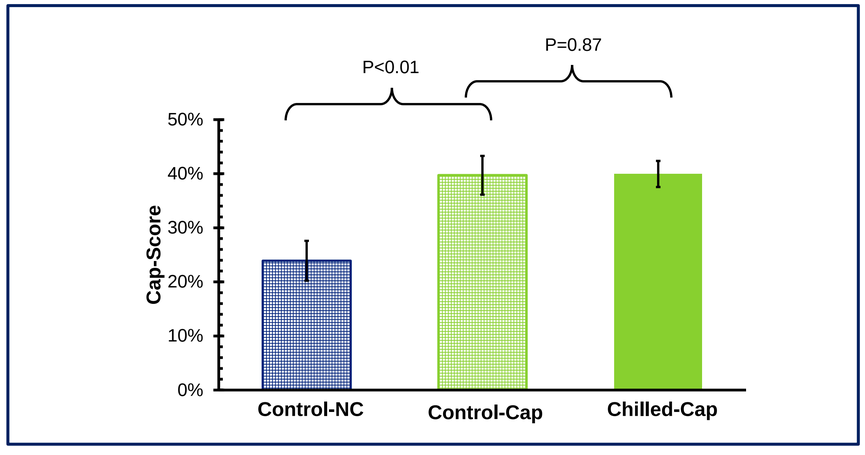


Figure 1. ***The effects of semen extension and cooling on Cap-Score™.*** Five samples were collected and split into control and chilled treatments (incubated with TYB and chilled overnight). Cap-Score was determined for control and chilled samples that were incubated with (Cap) or without capacitation stimuli (NC). The bar graph shows average Cap-Scores (y-axis) for each treatment (**x**-axis). An increase was observed in the Control-Cap when compared to the Control-NC treatment (40±4 vs 24±4%; p<0.01). There was no difference between the Control-Cap and the Chilled-Cap treatments (40±4 vs 40±2; p=0.87).

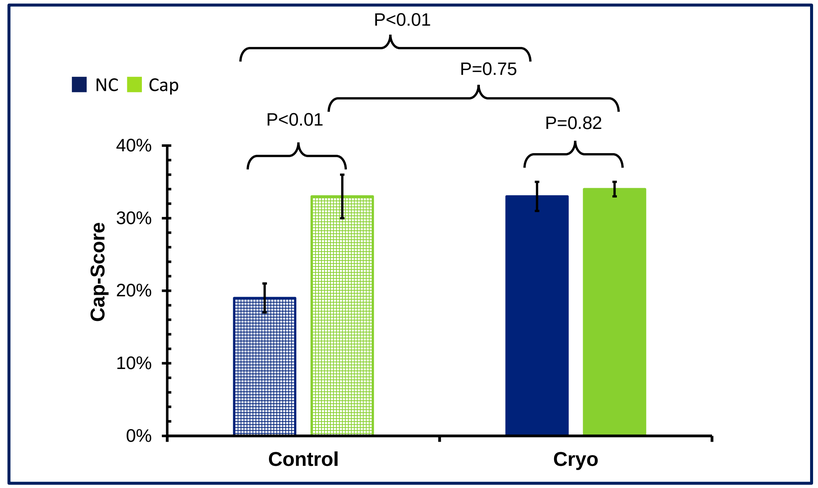


Figure 2. ***The effects of cryopreservation/thawing on Cap-Score™.*** Ten samples were collected and split; half of the fresh ejaculate was processed for Cap-Score (Control) and the other half was cryopreserved and processed for Cap-Score after thawing (Cryo). For both, Control and Cryo treatments, non-capacitated (NC) and capacitated (Cap) samples were prepared. The bar graph shows average Cap-Scores (y-axis) for each treatment (x-axis). An increase was seen in the control Cap over the control NC (33±3 vs 19±2; p<0.01). Cap-Score was unchanged for Cryo Cap when compared to control Cap (34±1% vs 33±3%; p=0.75). No difference was observed between the Cryo NC and Cryo Cap (33±3 vs 34±1; p=0.82). The Cryo NC was greater than the control NC (33±3 vs 19±2%; p<0.01).

Conclusions

- Semen extension in TYB and overnight maintenance at reduced temperature had no detectable impact on Cap-Score™.
- Cryopreservation/thawing induced capacitation-like membrane changes in sperm incubated under non-capacitating conditions.
- No difference in Cap-Score was observed between control and cryopreserved samples incubated under capacitating conditions.
- Being able to identify impacts on capacitation could assist in the optimization of protocols intended to preserve male fertility and improve IUI and IVF outcomes.

Funding