Sperm CryoProtecII™ - the ultimate chilling experience

Introduction

Essential prerequisites to maximize sperm survival during cryopreservation are an appropriate cryoprotective medium and a suitable protocol for freezing and thawing. NidaCon International has recently developed a new cryoprotective medium, ***Sperm CryoProtecII™*****,** which gives good results in comparison with other commercially available sperm freezing media. The objective of the present study was to optimize the cooling and freezing protocol to maximize the recovery rate of motile sperm after freezing and thawing.

Experiments and results

Experiment 1

Semen from volunteer donors was prepared on ***PureSperm®*** density gradients. The resulting sperm pellet was washed in ***PureSperm® Wash***. After removing most of the supernatant, the remaining 250 µL ***PureSperm® Wash*** was used to resuspend the sperm pellet. An equal volume of ***Sperm CryoProtecII™*** was added drop wise to the sperm suspension, ensuring thorough mixing between drops. Straws were filled with aliquots of the resulting sperm suspensions and were subjected to the following treatments:
i) straws were frozen in liquid nitrogen vapour immediately after sperm preparation;
ii) straws were equilibrated for 1 hour at room temperature before freezing in liquid nitrogen vapour;
iii) straws were equilibrated for 1 hour at 4-8° C before freezing in liquid nitrogen vapour.

After storage in liquid nitrogen for 48 hours, the straws were thawed by placing them in a water bath at 37°C for 30 seconds. Computer assisted sperm analysis was performed using a Hobson Sperm Tracker. The proportion of motile sperm recovered was compared for the different treatments (Figure 1). Equilibrating the sperm at 5°C before freezing resulted in a higher proportion of motile sperm on thawing than the other treatment protocols.

Experiment 2

An equal volume of ***Sperm CryoProtecII™*** was added drop wise to unprocessed ejaculates, ensuring thorough mixing between each addition. Straws filled with the sperm suspension were equilibrated at 4°C for 1 hour before freezing in liquid nitrogen vapour. The straws were stored, and subsequently thawed for sperm analysis as described in Expt.1. The results (Figure 2) showed that unprocessed human ejaculates can be frozen satisfactorily using ***Sperm CryoProtecII™*****.**

Figure 1: Effect of cooling prior to freezing on proportion of motile sperm post-thaw (n = 8).

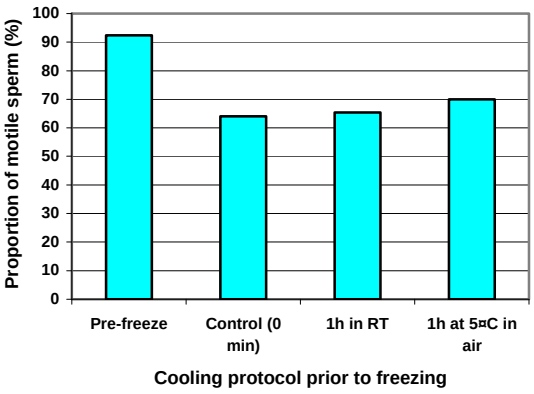
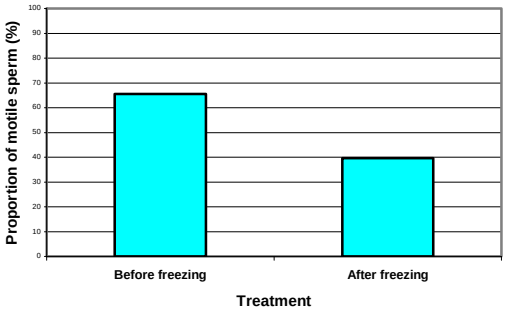


Figure 2: Freezing of unprocessed ejaculates with Sperm CryoProtecII™ (n = 9).



Conclusions

1. ***Sperm CryoProtecII™*** provides good survival of human sperm during freezing and thawing.
2. Equilibrating the sperm in ***Sperm CryoProtecII™*** for 1 hr. at 5°C before freezing improved sperm survival.
3. While unprocessed ejaculates can be frozen with ***Sperm CryoProtecII™*****,** maximal sperm survival is obtained by processing the semen on a PureSperm® gradient before freezing.

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