SPERM VITRIFICATION BY DROPPING DIRECTLY INTO LIQUID NITROGEN: AN ALTERNATIVE TO CONVENTIONAL CRYOPRESERVATION

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INTRODUCTION

Sperm cryopreservation is an important method to the management and preservation of male fertility which also has great potential for male infertility treatment as used in Assisted Reproductive Technology (ART). There are a variety of cryopreservation methods in order to preserve sperm in a long term. However, conventional methods have less effectiveness on severe oligozoospermia. The aim of this study is to compare the effectiveness of human severe oligozoospermia cryopreservation between slow cooling using Nicool LM-10 and microdrop vitrification.

Methods: We used 30 human severe oligozoospermia samples of 30 patients. Each semen sample was divided into 3: Group 1 cryopreserved by slow cooling using Nicool LM-10; Group 2 cryopreserved by microdrop vitrification with cryoprotectant agent (CPA); Group 3 cryopreserved by microdrop vitrification without CPA. For microdrop vitrification, 10 µl suspensions of cells were dropped directly into liquid nitrogen. The total sperm count, the retrieval rate, vitality, motility and morphology of each semen sample were assessed before and after freezing and thawing on Days 7 post freezing

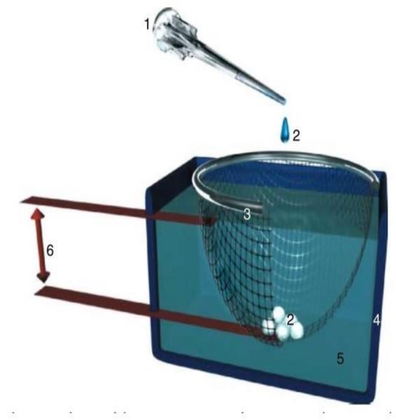

Figure 1. Scheme of the spermatozoa vitrification procedure

RESULTS

The revival rates of frozen sperm were low in all groups (32.57, 40.16, 35.64 respectively, p>0,05). But there were significantly highers in group 2 when compared with group 1 in the retrieval rate (90.73 vs 80.56, p<0.05) and the motility (46.14 vs 18.86, p<0.05). Besides, there were no significant differences between vitrification without CPA and slow cooling in all parameters.

Parameters	*Nicool LM10 (1) (X ± SD)*	*Microdrop vitification with CPA (2) (X ± SD)*	*Microdrop vitrification without CPA (3) (X ± SD)*	*p*
CSF (%)	32,57 ± 17,89	40,16 ± 20,60	35,64 ± 21,98	$p_{1/2}$; $p_{1/3}$; $p_{2/3}$ > 0,05
PR Motality CSF (%)	12,47 ± 14,98	38,13 ± 33,01	22,18 ± 28,77	$p_{1/2}$ <0,05 $p_{1/3}$ >0,05 $p_{2/3}$ <0,05
Motality CSF (%)	18,86 ± 15,08	46,14 ± 31,48	24,59 ± 22,32	$p_{1/2}$ <0,05 $p_{1/3}$ >0,05 $p_{2/3}$ <0,05
Retrieval rate (%)	80,56 ± 22,24	90,73 ± 16,74	89,87 ± 17,46	$p_{1/2}$ <0,05 $p_{1/3}$ <0,05 $p_{2/3}$ >0,05

Table 1. Comparison CSF parameters before and after cryopreservation of 3 groups (n=30)

CONCLUSION

Sperm parameters were significantly reduced after cryopreservation but highest results were observed for the microdrop vitrification with CPA. Microdrop vitrification has great potential to cryopreserve a low number of sperm. It may be a simple, safe and effective method for the cryopreservation of severe oliozoospermia semen. Otherwise, human sperm can also be vitrified quickly and simply without CPA with its possible toxicity.

REFERENCES

1. E Isachenko, V Isachenko, J M Weiss, et al. (2008). Acrosomal status and mitochondrial activity of human spermatozoa vitrified with sucrose. *Hum Reprod*, 136, 167-173.
2. Agha-Rahimi A., Khalili M.A., Nabi A., et al. (2014). Vitrification is not superior to rapid freezing of normozoospermic spermatozoa: effects on sperm parameters, DNA fragmentation and hyaluronan binding. *Reprod Biomed Online*, 28(3), 352-358.
3. Sánchez R. (2012). Vitrified sperm banks: the new aseptic technique for human spermatozoa allows cryopreservation at -86°C. *Andrology*, 44, 433-435.
4. JK Sherman (1990). Cryopreservation of human semen. *CRC Handbook of the Laboratory Diagnosis and Treatment of Infertility*, *CRC Press, Boca Raton*, 229-259.
5. Jin L1, Zheng JF, Liu Q, Ren XL, Hu J, Wei YL (2010). Microdrop-vitrification for epididymal spermatozoa without cryoprotectants. *Zhonghua Nan Ke Xue*, 16(12), 1089–1094.

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