A comparative gene expression analysis of slow frozen and vitrified metaphase II human oocytes

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Introduction
Routine application of oocyte cryopreservation procedures for both long and short-term fertility preservation and treatment has been widely accepted. Several modifications in the processes of vitrification and slow oocyte freezing have significantly improved the survival rates, pregnancy and live-birth rates obtained from both procedures. However, vitrification seems to produce better results than slow oocyte freezing. Unfortunately, the safety of these procedures and their impact on oocytes at the molecular level is not completely understood.

To investigate the impact of metaphase II (MII) human oocyte cryopreservation by slow freezing and by vitrification on the expression of genes involved in oocyte cryo-survival and in-vitro developmental competence.

Aim

Material and Methods
A total of 15 patients in the vitrification group had controlled ovarian stimulation between May and October 2012. A sibling pair of unfractured MII oocytes was obtained from each patient in a fresh ICSI cycle, of which one was vitrified, using a closed system and one was sham treated as a paired control. Two fertility preservation patients in the slow freezing group had 21 oocytes cryopreserved by slow freezing between 2002 and 2003. All cryopreserved oocytes were thawed and survival rates were compared between groups. cDNA was obtained using poly(A)PCR amplification and quantified using a picogreen assay and subsequently analysed by qPCR. Statistical analysis was by Fisher’s exact and two way ANOVA test with Bonferroni post test. P values <0.05 were considered significant. All oocytes were obtained with informed written patient consent with approval from a local ethics committee and under HFEA Research Licence R0026

Results
The average ages of patients in the slow freezing, vitrification and control groups were 31.4yrs, 32.8yrs and 32.8yrs, while the survival rates were 76.2% and 85.7% in the two study groups respectively (not statistically significantly different ; p=0.6897). Gene expression analysis of BRCA1 and 2, KIF2, OCT4, TUBB4Q and Beta-actin (as the house keeping gene) showed an increase in the expression levels of BRCA1 and 2, KIF2 and TUBB4Q in the slow freezing group compared with the vitrification and control groups respectively. Statistically significant increases were only seen in the expression of BRCA1 and KIF2 genes in the slow freezing group, p=0.001. Conversely OCT4 and TUBB4Q both tended to be down-regulated in the slow freezing and vitrification groups respectively when compared with expression profiles of the study control, however, this was not statistically significant.

Conclusion
Our findings show that oocyte cryopreservation by both vitrification and slow freezing may be associated with altered gene expression profiles. These methods may allow us to test the effectiveness and safety of cryopreservation methods especially in women undergoing fertility preservation.

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