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Oral Presentations

O-5
Genetics and transcriptome profile of cryopreserved human sperm associated antigens (SPAGs)

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Background: Human sperm associated antigens (SPAGs), formerly known as sperm membrane protein, are eighteen-type proteins that some types of them, have a momentous role in various biological functions especially fertility outcome. The molecular weight range allotted to SPAG proteins is between 24-71 k Da. The role of 8 types of these SPAGs (SPAG 1, 2, 6, 8, 9, 12, 13, 15) has been confirmed in infertility. Thus, any damage in quoted SPAGs can lead to infertility. In spite of favorable aspect of cryopreserved sperm for assisted reproductive technology, detrimental impact of freezing on cells has been quoted in many studies. In this regard, cryopreservation has an unfavorable effect on sperm quality perhaps via perturbation of SPAGs expression.

Objective: This study aimed to appraise the impact of rapid freezing on the gene expression of SPAGs in normal human spermatozoa.

Materials and Methods: The semen samples were collected from 12 normospermic individuals. All twelve normo-ejaculated samples were prepared via density gradient centrifuge and thereupon, the aliquots of motile sperms were divided into two fresh and freeze groups. Afterwards, sperm samples were mixed (1:0.7) with spermfreeze® cryoprotectant for 10 minutes. Then the mixture was loaded into cryotube and freezed with rapid freezing procedure. After three days of freezing at -196°C, the specimen were thawed in tap water for 5 min and incubated for 2 hr at recovery time in a CO_2 incubator. RNA from sperm was extracted with TRIzol. After synthesis of *cDNA*, *SPAGs* gene expression analysis was performed using Real-time PCR.

Results: The results of statistical analysis showed a decrease in the gene expression of SPAG5, SPAG7, SPAG12 (SNU13/ NHP2L1) during rapid freezing procedure. The results are significant at the $p \leq 0.05$ level. No significant reduction in the expression level between fresh and freeze group was found in remained SPAGs.

Conclusion: The results pointed out that cryopreservation procedure could negatively affect gene expression of some SPAGs in human spermatozoa. Hence, alteration of SPAGs expression could offer new suggestions to evaluate probable molecular correlations between freezing and increased failure rate of in vitro fertilization and intracytoplasmic sperm injection.

Key words: *Antigen, Cryopreservation, Fertility, Human, Sperm.*