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LOOKING FOR MOLECULAR BIOMARKERS OF CRYODAMAGE IN DONORS SEMEN.

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OBJECTIVE: Sperm cryopreservation is a widespread tool in assisted reproduction for the management of male infertility and it is mandatory for sperm donor programs. It is well-established that cryopreservation causes loss of sperm motility, impacting on its reproductive competence. Therefore, the selection of sperm donor samples with good freezability and fertilization abilities is important for optimization of sperm banks. The molecular basis of the vulnerability to cryopreservation is still unknown. Microarray provides a powerful tool to explore the molecular mechanisms involved in cryoinjury. The objective of this study is to characterize the gene expression profiles of semen samples according to their resistance to cryopreservation and to determine the impact of cryodamage on pregnancy outcomes after donor intrauterine insemination (IUI).

DESIGN: Prospective cohort study using sperm samples from 28 donors attending IVI Alicante from 2014 to 2017.

MATERIALS AND METHODS: Donor sperm samples were categorized according to their high resistance (HR, n ¼ 14; >0.5988 posthaw/fresh motility) or low resistance (LR, n ¼ 14; <0.5988 posthaw/fresh motility) to cryopreservation. The mRNA was extracted with the QIAgen kit and analyzed on Agilent Bioanalyzer 2100. Whole genome microarray was individually performed and results were analyzed to detect genes differentially expressed. Limma moderated T-Statistic was used to analyze differential expression ($P < 0.05$). Functional analysis was performed using Gene Ontology (GO) bioinformatics tools to determine differences in tree GO terms: biological processes, molecular functions and cellular components. For cryodamage correlation with pregnancy success in IUI 16 donor's samples were employed (6 HR; 10 LR).

RESULTS: Gene expression: No differentially expressed genes were observed between HR and LR samples among the 19619 genes analyzed. Functional analysis: Eighty six from the 5648 biological processes analyzed were found significant different between HR and LR samples (13 up-regulated (U) and 73 down-regulated (D)). In addition, significant differences were observed between those groups in 39 cellular component GO terms (3 U and 36 D) and in 2 molecular functions GO terms (1 U). Clinical outcomes: Up to now, 24 and 37 IUI have been performed with samples from HR and LR group, obtaining 9 and 13 pregnancies, respectively. Apparently, the use of samples with better freezability in IUI seem to slightly increase pregnancy success. However, differences are not significant.

CONCLUSIONS: Although no gene was found to be differentially expressed, significant changes in several biological functions were observed between samples with different

freezability, probably due to the sample size Table 1: Patient age, oocyte number, number MII, fertilization and pregnancy rates FNA-Fresh Micro-TESE Fresh Micro-TESE Frozen MESA Fresh MESA Frozen Fresh Ejaculate Frozen Ejaculate n 84 20 215 6 17 1243 34 F Age 35.5 _ 6.7 33.2 _ 4.7 32.4 _ 5.9 31 _ 7.0 34.5 _ 5.1 32.3 _ 4.9 33 _ 5.8 M Age 46.5 _ 16.5 37.4 _ 5.8 38 _ 9.0 38 _ 5.8 42.8 _ 13.0 38 _ 7.2 37.8 _ 6.4 Oocytes 10.4 _ 5.9 14.1 _ 10.3 11.2 _ 7.3 11 _ 6.4 12.9 _ 7.8 12.2 _ 8.7 10.0 _ 5.4 MII 7.6 _ 4.9 10.6 _ 8.3 7.8 _ 5.5 9.3 _5.9 8.6 _ 6.1 8.5 _ 6.8 7.1 _ 4.1 Fertilized 4 _ 3 6.1 _ 5.1 3.7 _ 3.1 6.5 _ 4.5 6.6 _ 4.1 6.3 _ 4.9 4.5 _ 3.6 Fertilization % 50 51.3 43.4* 64 65.1 67.5 56.5 Pregnancy % 37* 67 35* 67 64 44* 48* FERTILITY & STERILITY_ e295 analyzed. In the other hand, there were no significant differences between pregnancy results. More arrays analysis and IUI are necessary to confirm the results. Supported by: Project supported by the Center for the Development of Industrial Technology (CDTI no IDI-20141242.) from the Spanish Ministry , co-financed by the European Regional Development Fund (ERDF).