

DIFFERENTIAL SPERM mRNA EXPRESSION PROFILE FROM FERTILE VS INFERTILE MALES: A PRELIMINARY MICROARRAY APPROACH OF SPERM EXPRESSION PROFILES (SEP) IN FERTILITY. N Garrido JA Martínez-Conejero, J Jauregui, JA Horcajadas, A Agarwal, J Remohi, A Pellicer, M Meseguer

**Introduction:** Sperm analysis based on sperm count and motility has been employed for the diagnosis of male fertility for several decades. It is an easy, inexpensive and useful tool to determine the fertile status of a male. A significant number of infertile males are diagnosed with idiopathic infertility and all sperm parameters are normal. Recent investigations have described the sperm mRNA relevance in fertilization and early embryo development. Microarray technologies can inform about a wide range of mRNAs expression within a single experiment, and are ideal in analyzing the expression profiles in cells or tissues. Our aim was to compare the sperm expression profiles obtained from infertile males and compare with the fertile sperm donors by employing microarray technology and determine the differentially expressed genes that may be potentially involved in male fertility.

**Material and methods:** Sperm samples were obtained from infertile males (n=5) and proven fertile donors (n=5) with normal sperm count and motility (WHO criteria). Sperm mRNA was extracted using Trizol<sup>®</sup> and resuspended in DEPC-treated water and frozen at -80 until the microarray experiments were performed. RNAs from the same groups were pooled before the analysis. Human whole Genome bioarray contains more than 55,000 gene targets. Comparisons between the two groups were performed in duplicate. Intensities were normalized and analyzed using CodeLink Expression Analysis v4.1 software.

**Results:** Only differentially expressed genes that were expressed at least ten times compared with the control group were included. Our preliminary results confirm that within the differentially expressed genes there are few genes that are overexpressed (n=3), while all others are underexpressed (n=133) in infertile males.. Our results suggest that failure to impregnate a woman with normal sperm production may be due to the lack of factors involved in correct sperm function.(Figure 1). Negative results denote a higher expression in the control group. Among all the gene sequences found to be differentially expressed, it is notable to remark the presence of several ribosomal proteins and factors involved in spermatogenesis. Interestingly, those spermatogenesis factors are not related with sperm production in terms of number of ejaculated spermatozoa given that all the samples obtained for his analysis were within normal range of sperm count and comparable in both groups.

**Conclusions:** This is the first time that a significant difference in the mRNA sperm expression profiles in spermatozoa obtained from infertile males vs spermatozoa obtained in fertile men with comparable sperm count has been described. These differences imply several molecules in a broad spectrum of biochemical and physiological pathways, at different points. These results confirm the complexity of the events involved in sperm function.

**Comment [j1]:** Yo lo cambiaría a: given the huge amount of data, only those genes with a fold change over ten fold were considered differentially expressed. JOSON

**Comment [j2]:** Esa frase es muy larga y no se entiende bien. JOSON