

P-554 Wednesday, October 21, 2015

## WITHDRAWN

P-555 Wednesday, October 21, 2015

**MORPHOLOGICALLY DISTINCT PHENOTYPES OF SPERMATOZOA IN INFERTILE MEN REVEAL DOWN REGULATION OF MULTIPLE SIGNALING PATHWAYS.**

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**OBJECTIVE:** Seven morphologically distinct spermatozoal phenotypes can be detected in human semen under electron microscopy: sperm with dysplasia of the fibrous sheath, non-specific flagellar defects, immotile cilia syndrome, acrosomal hypoplasia, defective chromatin condensation and compaction, pin head and sperm without heads. These conditions cannot be identified by routine semen analysis because they are but secondary manifestations of underlying pathology. We hypothesize that proteomics would enable us to better understand the mechanism(s) underlying these pathologies.

**DESIGN:** This proteomic study analyzed proteins in spermatozoa from infertile men fractionated on three layers of density gradient (80, 60 and 40%). Fraction 1 (F1) refers to the least mature stage having the lowest density whereas fraction 4 (F4) contains the most dense and morphologically mature motile spermatozoa. Fraction 2 (F2) and fraction 3 (F3) are the intermediate stages.

**MATERIALS AND METHODS:** 1-D gel electrophoresis followed by LC/MS-MS (LTQ-Orbitrap Elite hybrid mass spectrometer) was used for protein identification. Mascot (Matrix Science, London, UK), SEQUEST (Thermo Fisher Scientific, San Jose, CA, USA) and X! Tandem (TheGPM, [thegpm.org](http://thegpm.org)) were set up to search the human reference with database assuming trypsin as the digestion enzyme. Functional annotations of proteins were obtained using bioinformatic tools and pathway databases.

**RESULTS:** A total of 1202 proteins were identified in the F1 fraction while 1140, 1025 and 890 proteins were recovered from the three other fractions, F2, F3 and F4 respectively. With respect to the differentially expressed proteins, F1 exhibited the highest number (522), followed by F2 (362) and lowest was observed in F3 (188) as compared to F4. Ingenuity Pathway analysis revealed 162 pathways showing a decreasing trend from F1 through F4. The principal pathways were spermatogenesis, protein metabolism, cell cycle, integration of energy metabolism, regulation of apoptosis, cell redox homeostasis and translational elongation. The proteins detected in our data set belonging to the top 15 pathways shared many proteins that were downregulated, suggesting failure of signaling cascade in the process of maturation in infertile patients.

**CONCLUSIONS:** We conclude that a defective signaling cascade is responsible for the faulty sperm function in infertile patients. Particularly, a decline in mitochondrial function and oxidative phosphorylation in the F4 fraction implies an energy deprived hypoxic state. Dysregulated protein turnover and protein folding may lead to accumulation of defective proteins and can potentially impair of sperm function.

P-556 Wednesday, October 21, 2015

**A COMPREHENSIVE ASSESSMENT OF HISTONE MODIFICATIONS IN HUMAN SPERM.** S. B. Schon,<sup>a</sup> L. J. Luense,<sup>b</sup> X. Wang,<sup>b</sup> G. Donahue,<sup>b</sup> B. A. Garcia,<sup>b</sup> M. S. Bartolomei,<sup>b</sup> S. L. Berger.<sup>b</sup> <sup>a</sup>Reproductive Endocrinology & Infertility, University of Pennsylvania, Philadelphia, PA; <sup>b</sup>University of Pennsylvania, Philadelphia, PA.

**OBJECTIVE:** The unique process of spermiogenesis involves a dramatic reorganization of paternal chromatin, whereby ~90% of histones are evicted and replaced with protamines. Proper exchange is critical for nuclear compaction and abnormalities in this process have been associated with male infertility [1,2]. Recent evidence suggests that the ~10% of histones retained in mature sperm are located at specific genomic loci. Furthermore, selective histone modifications (i.e. acetylation, methylation) are enriched at genes encoding master regulators of embryo development, suggesting a role for paternal transmission of epigenetic information [3,4]. To date, our understanding of histone modifications in human sperm has largely been limited to descriptive data of specific modifications. Our objective was to perform a comprehensive evaluation of histone modifications in normal sperm, to quantify their abundance, and to assess variation in modifications between individuals and in fresh vs. frozen sperm.

**DESIGN:** Descriptive study of human sperm with normal semen analysis parameters utilizing acid extraction of histones and bottom up mass spectrometry to identify and quantitate histone modifications.

**MATERIALS AND METHODS:** Normal semen samples were obtained from men undergoing routine semen analysis (n=8). 4 samples were equally divided with one portion cryopreserved at our Andrology Laboratory and one half processed immediately. Following acid extraction, histones were digested into peptides for bottom up LC-tandem mass spectrometry by treatment with propionic acid and trypsin. The identities and relative abundance of peptides and associated modifications were analyzed based on retention time, MS1 and MS2 of ion peaks. Kruskal-Wallis and ANOVA tests were used as appropriate for assessment of inter-individual variability in modification abundance and to compare fresh vs. frozen samples.

**RESULTS:** We identified modifications on all four canonical histones (H2A, H2B, H3, H4), the linker histone H1 and histone variants. We describe a total of 102 modifications identified for the first time in human sperm. Variability in modifications on H3 was noted between individuals, while the abundance of modifications on H4 was consistent. No differences were observed between fresh vs. frozen sperm.

**CONCLUSIONS:** Our study is the first to provide a comprehensive and quantitative analysis of histone modifications in human sperm. We demonstrate significant variation between individuals in specific modifications on H3 and relative conservation in the abundance of H4. The conservation of specific modifications further supports the hypothesis that the paternal epigenome plays a role in early developmental programming and we further postulate that aberrations in histone modifications may play a role in decreased fertility. Future analysis will focus on modification profiles of abnormal sperm.

## References:

1. Carrell, D.T. and L. Liu, Altered protamine 2 expression is uncommon in donors of known fertility, but common among men with poor fertilizing capacity, and may reflect other abnormalities of spermiogenesis. *J Androl*, 2001. 22(4): p. 604-10.