ABSTRACT

Objective: Seminal plasma is responsible for the nourishment, protection, metabolic and endocrine functions of spermatozoa within the female reproductive tract. Reactive oxygen species (ROS) play a major role in the pathophysiology of male infertility by modulating sperm function. Seminal plasma is a natural resource of antioxidants and protects against ROS toxicity. The goal of this study was to investigate ROS-mediated expression profile of proteins in seminal plasma of infertile patients with different ROS levels.

Design: A prospective comparative proteomic study

Materials and Methods: Seminal plasma from fertile (control group) and infertile men with varying levels of ROS was assessed to identify significant proteins involved in ROS-mediated reproductive dysfunctions. Infertile men were stratified into two subgroups into categories of low or high ROS (≤ 93 RLU/sec/10⁶ sperm; n = 11), medium ROS (>93-500 RLU/sec/10⁶ sperm; n = 17) and high ROS (>500 RLU/sec/10⁶ sperm; n = 14) and compared with fertile men (<93 RLU/sec/10⁶ sperm; n = 17). ROS levels were measured in the seminal ejaculates by chemiluminescence assay. 1-D gel electrophoresis followed by LC/MS/MS was used for protein identification. Mascot, SEQUEST and X! Tandem were used to search the human reference with database assuming trypsin as the digestion enzyme. Functional annotations of proteins were obtained using bioinformatics tools and pathway database.

Results: Semen analysis revealed that proteins belonging to extracellular regions were differentially regulated in all three infertile patient groups compared to the fertile controls. Molecular mechanisms underlying this regulation were further explored in a more comprehensive set of experiments, which involved quantification of the abundance and expression level of proteins. Alterations in relative abundance were observed, resulting in a decrease in proteins of extracellular origin with increase in ROS levels compared to fertile donors. This was consistent across different categories of ROS.

Conclusions: The expression of MME and FAM3D along with ROS level in the seminal plasma may serve as good markers for diagnosis of male infertility.

INTRODUCTION

Seminal plasma contains secretions that are derived from the testis, epididymis and accessory glands, including the prostate, seminal vesicles and Cowper’s gland. A considerable volume of research has focused on the mechanism(s) involved in production, morphology and nourishment, protection, capacitation, and motility of sperm within the female reproductive tract. The function of spermatozoa, however, the critical role that seminal fluid plays in imparting motility and fertilizing a progesterone-primed oocyte. The effects of ROS on different areas of the male reproductive system and male infertility have been described in several studies. Reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide, are released from activated leukocytes and the vascular endothelium.

RESULTS

Global Expression profile of proteins involved in sperm motility (Figure 1). The expression of proteins involved in sperm motility was affected by the levels of ROS. The most intensely upregulated proteins were those involved in sperm motility and fertilization (Figure 2).

Proteomic signatures of seminal plasma in infertile men with different levels of ROS. The distribution of overexpressed and underexpressed proteins in each of the three categories (i.e., Low, Medium and High ROS) and control group is shown in Figure 3.

Function annotation and pathway analysis of differentially expressed proteins. The distribution of overexpressed and underexpressed proteins in each of the three categories (i.e., Low, Medium and High ROS) and control group is shown in Figure 4.

Proteomic signatures of seminal plasma in infertile men with different levels of ROS. The distribution of overexpressed and underexpressed proteins in each of the three categories (i.e., Low, Medium and High ROS) is shown in Figure 5.

CONCLUSIONS

1. We have for the first time demonstrated the presence of S35 of a single pathway that may lead to impairment of sperm function in men with low, medium or high ROS levels.

2. NWE and FAM3D along with ROS level in the seminal plasma may serve as good markers for diagnosis of male infertility.