IPD Project Details

Project ID: IPD1849

Project Title: Viral fallout: Semen proteome reveals COVID-19 dysregulates several biological processes linked to male reproductive function

Description: The current global outbreak of COVID-19 has significantly impacted several organ systems. Male reproductive organs are among the potential targets of SARS-CoV-2 infection owing to the presence of abundant viral receptors; ACE2 and TMPRSS2 in the testis. However, the questions pertaining to the long term effects of SARS-CoV-2 infection on male fertility are still unanswered. For this study, we procured the semen samples of COVID-19 recovered patients and performed mass spectrometry based proteomic studies to understand the impact of the disease on the biological processes involved in reproductive health.

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Sample Preparation: Semen samples were obtained by masturbation into sterile containers after about three days of abstinence. After collection, samples were kept at 37°C for 30 minutes for liquefaction before analysis and then taken forward for semen parameter assessment. Initially, from the semen samples, the proteins were precipitated with ethanol, followed by sonication in urea lysis buffer. Then, centrifugation was done at 8000 g for 15 mins, and the clear supernatant was collected. Finally, the protein extracts were sent for analysis at MASSFIITB (Mass Spectrometry Facility at IIT Bombay). The protein was quantified using Bradford assay, and 30 µg of the protein was taken forward for digestion. Before tryptic digestion, the protein was reduced with Tris carboxyethyl phosphine (TCEP) with a final concentration of 20 mM for 1 hour at 37°C and alkylated Iodoacetamide (IAA) with a final concentration of 40 mM for 15 mins in the dark.

Peptide Separation: The digested peptide was desalted to remove any contaminants using a C-18 stage tip. The peptides derived after the desalting procedure were vacuum dried, reconstituted in Milli-Q water with 0.1 % of formic acid, and quantified using the Scopes method. Finally, one µg of the peptide was injected for the liquid chromatography-tandem mass spectrometry (LC-MS/MS) run. For Liquid Chromatography-Tandem Mass Spectrometry, 1µg peptide of each sample was run in

Orbitrap Fusion Tribrid Mass Spectrometer (Thermo Fischer Scientific) with easy nano LC 1200 system with a gradient of 80% ACN and 0.1% FA for 120 min with blanks after every sample. BSA was run at starting and end point of each set of run to check the instrument quality. All samples were loaded onto the LC column at a flow rate of 300nl/min. Mass spectrometric data acquisition was done in data dependent acquisition mode with a mass scan range of 375-1700 m/z and mass resolution of 60,000. A mass window of 10ppm was set with a dynamic exclusion of 40s. All MS/MS data was acquired by High energy Collision Dissociation method of fragmentation and data acquisition was done using Thermo Thermo Xcalibur software version 4.0.

Protein Characterization: The mass spectrometric raw datasets were processed using Label-Free Quantification based parameter in MaxQuant(18) against Uniprot human database (downloaded on March 11, 2021) release date 10.02.2021 (first release of 2021), searched with a built-in search engine- Andromeda which contains total 1099 proteins. Trypsin was set as the enzyme for digestion with maximum permissible missed cleavage as 2. Carbamidomethylation at cysteine was set as static modifications, and Oxidation at methionine was kept as variable modification. Thus, to increase the reliability of data obtained, proteins having a maximum of 1% false discovery rate (FDR) and unique peptides greater than 1 were selected.

Experiment Type: Shotgun proteomics

Species: Homo sapiens -9606

Tissue: Semen (bto:0001230)

Cell Type: Sperm (cl:0000019)

Disease: Covid-19 (doid:0080600)

Instrument Details: Orbitrap Fusion (MS:1002416)

Protein Modifications: monohydroxylated residue, acetylated residue, iodoacetamide derivatized residue

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