**EFFECT OF OXIDATION-REDUCTION POTENTIAL ON MITOCHONDRIAL MEMBRANE POTENTIAL AND VITALITY OF PHYSIOLOGICALLY NORMAL HUMAN SPERMATOZOA.** Manesh Kumar Panner Selvam, PhD, Ashok Agarwal, PhD, Renata Finelli, PhD, Christopher M. Douglas, B.A., M.S., Ralf Henkel, PhD, Sajal Gupta, MD, Rakesh Sharma, PhD. American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, University of the Western Cape, Bellville, South Africa.

OBJECTIVE: Physiological levels of reactive oxygen species (ROS) are necessary for optimal sperm functions such as total and progressive motility. In our previous study, we have demonstrated that higher levels of seminal oxidation-reduction potential (ORP) negatively affects total and progressive motility. Furthermore, motility is directly related to sperm vitality and mitochondrial membrane integrity. The objective of the present study was to investigate the effect of ORP on vitality and mitochondrial membrane potential (MMP) of physiologically normal spermatozoa.

DESIGN: Physiologically normal sperm from donor semen samples (n=8) were exposed to different titrated levels of oxidative stress (ORP: 1.48 and 2.75 mV/10^6 sperm/mL) in sperm wash medium (SWM). MMP and sperm vitality were measured at different time intervals (0, 60 and 120 minutes). The sample size for this study was calculated with an 80% power and a significance of P<0.05.

MATERIALS AND METHODS: ORP of SWM was taken as baseline (control) and the different ORP levels (1.48 and 2.75 mV/10^6 sperm/mL) were generated by titrating SMW with defined concentrations of the oxidative stress inducer, cumene hydroperoxide. Equal concentrations (≈20 μM) of JC-1 or MitoTracker Red fluorescence double-density gradient centrifugation-selected sperm (motility >90%) were incubated in SWM with different ORP levels for up to 120 minutes. Eosin-nigrosin staining was performed to evaluate the vitality; whereas, JC-1 dye was used to stain the sperm cells (≈1 x 10^6) to evaluate the depolarization of mitochondrial membrane. MMP was analyzed using flow cytometry after 60 and 120 minutes. Pairwise comparison analysis was carried out to determine the statistical significance.

RESULTS: MMP remained unchanged after sperm exposure for 60 minutes. MMP decreased to 2.5% (P=0.0014) and 61.1% (P<0.0001) at 120 minutes when sperm was exposed to ORP values of 1.48 mV/10^6 sperm/mL and 2.75 mV/10^6 sperm/mL, respectively. Vitality decreased to 21.2% (P=0.0001) at 60 minutes and 41.1% (P<0.0001) at 120 minutes when sperm were exposed to ORP values of 2.75 mV/10^6 sperm/mL.

CONCLUSIONS: The current findings demonstrate that spermatozoon MMP and vitality were affected at ORP levels of ≥1.48 mV/10^6 and ≥2.75 mV/10^6 sperm/mL, respectively. Hence, high seminal ORP may have a negative effect on sperm functionality and therefore on the fertilizing ability of spermatozoon.

Reference: None.

SUPPORT: None.

**COMPARATIVE PROTEOMIC ANALYSIS REVEALS DIFFERENTIAL REGULATION OF REDOX HOMEOSTASIS AND PURTURBED OXIDATIVE PHOSPHORYLATION PATHWAY IN UNILATERAL COMPARED TO BILATERAL VARICOCELE CONDITION.** Manesh Kumar Panner Selvam, PhD, Ashok Agarwal, PhD, Luna Samanta, PhD. American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, Redox Biology Laboratory, Department of Zoology, Ravenshaw University, Cuttack, India.

OBJECTIVE: Oxidative stress is pronounced in varicocele patients and differs between unilateral and bilateral conditions. At subcellular level, excess of oxidative stress induces damage to the cell organelles and plasma membrane. The main objective was to have a proteomic insight into seminal plasma for delineating the possible pathways involved in the etiology of sperm dysfunction in unilateral and bilateral varicocele condition.

DESIGN: Proteomic profiling of seminal plasma (unilateral varicocele, bilateral varicocele and fertile healthy men) was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Bioinformatic analysis was conducted using ingenuity pathway analysis (IPA) software.

MATERIALS AND METHODS: Pooled seminal plasma samples from unilateral (n=5), bilateral (n=5) varicocele patients and fertile healthy men (n=5) were subjected to quantitative proteomic analysis. Proteins identified by LC-MS/MS in both varicocele groups were compared separately and also as combined varicocele group with the fertile group. Differentially expressed proteins (DEPs) obtained from three different analysis were subjected to comparison analysis using IPA software.
OBJECTIVE: Cigarette smoking is one of the most important lifestyle-associated factors involved in human diseases, and both men and women who smoke present increased rates of infertility. We have previously shown that smoking leads to an altered seminal plasma proteome, with increased inflammatory mechanisms and immune function. We hypothesize that this inflammatory pathway in these men. This is an important finding because it could lead to targeted therapeutics based on this inflammatory aspect. However, it should be noted that seminal plasma is produced by fluid of various sources, so that identifying its tissue of origin could assist in understanding how smoking affects male fertility potential. Also, because there is an important exchange of information between the spermatozoa and the epididymis cells, it is of interest to demonstrate how inflammation distorts this exchange, and what the consequences are.

S100A9, AN INFLAMMATORY AND IMMUNE PROTEIN, IS INCREASED IN SEMEN OF SMOKERS. Mariana Pereira Antoniassi, PhD,a Larissa Berloffa Belardin, BSc, MSc,b Paula Intasqui, PhD,b Mariana Camargo, PhD,a Ricardo P. Bertolla, DVM, PhD a Sao Paulo Federal University, Sao Paulo, Brazil; bUniversidade Federal de Sao Paulo, Sao Paulo, Brazil; aHead of Research UNIFESP, SÃO PAULO, Brazil.

OBJECTIVE: Cigarette smoking is one of the most important lifestyle-associated factors involved in human diseases, and both men and women who smoke present increased rates of infertility. We have previously shown that smoking leads to an altered seminal plasma proteome, with increased inflammatory mechanisms and immune function. We hypothesize that this inflammatory pathway in these men. This is an important finding because it could lead to targeted therapeutics based on this inflammatory aspect. However, it should be noted that seminal plasma is produced by fluid of various sources, so that identifying its tissue of origin could assist in understanding how smoking affects male fertility potential. Also, because there is an important exchange of information between the spermatozoa and the epididymis cells, it is of interest to demonstrate how inflammation distorts this exchange, and what the consequences are.

SUPPORT: None.

RESULTS: Smenal plasma proteomic analysis revealed the presence of cellular proteins particularly of mitochondrial origin in seminal plasma. Proteins involved in the oxidative phosphorylation pathway of spermatozoa were present (Z score = -3.5) in unilateral varicocele patients. Whereas, the Z-score was not available for combined varicocele and bilateral varicocele groups (Table 1). In addition, proteins regulating the cellular antioxidant mechanism such as SOD1 (Z score = 3.94) and SOD2 (Z score = 8.08) were detected in unilateral varicocele patients. Whereas, IL-8 signaling pathway was activated in bilateral varicocele group (Z score = 2.236) compared to unilateral varicocele group (Z score = 1.34).

CONCLUSIONS: Our proteomic result implies release of spermatozoal proteins into seminal plasma of unilateral varicocele patients may be due to oxidative damage of sperm membrane or inflammation originating from mitochondrial dysfunction. On the other hand, in case of bilateral varicocele it may due to apoptosis which might have been phagocytized thereby, no cellular content is released into seminal plasma.

Reference: None.

SUPPORT: None.

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EXOME SEQUENCING IDENTIFYING ADDITIONAL QRICH2 MUTATIONS IN OLIGO-ASTHENO-TERATOZOOSPERMIA AND ASTHENOSPERMIA PATIENTS. Wenming Xu, Ph.D. Xiao Liang Li, M.S., American Society of Human Genetics, Chengdu, China.

OBJECTIVE: Our recent study has shown that loss-of -function of QRICH2, a testis specific expressed gene, is associated with male infertility with multiple morphological abnormalities of the flagella (MMAF), the current study aim to determine whether QRICH2 mutations were associated with other more common forms of male infertility, such as oligo-astheno-teratozoospermia and asthenospermia

DESIGN: Experimental study recruited from male infertility clinic and human samples of case and control were collected.

MATERIALS AND METHODS: 94 cases of male infertility patients were recruited. WES was performed for all subjects. All identified variants were confirmed by Sanger sequencing. Immunostaining was used to determine the specific localization of QRICH2 in human sperm. Western blot were used to detect the expression of QRICH2 in oligo-astheno-teratozoospermia . Co-Immunoprecipitation (Co-IP) with QRICH2 antibody in human testis and proteomics analysis were conducted to identify the binding partner. IVF/ICSI outcome were followed to determine whether the mutation of QRICH2 have effect on the normal development of offspring.

RESULTS: We identified five unrelated patients (5/84, 5.9%) with homozygous and compound heterozygous mutations in the QRICH2 gene, which is specifically expressed in human and mouse testis. Three of the samples harbor a recurrent deletion, (g.17:74288566_74288568del.e1.1742_1744del.p.581_582del) .None of these mutations were reported in control sequence databases. 4 of mutation is located in the SNC-N domain, while one mutation is located in the Glutamine rich domain. Co-IP result indicated that mitochondrial proteins, such as VDAC1 is associated with QRICH2 . Western blot result shows that QRICH2 expression is -down-regulated in patients. And IVF/ICSI outcome analysis indicates that normal offspring development could be observed in the patients.

CONCLUSIONS: Compared with other reported genes associated with male infertility , high frequency of QRICH2 mutations were detected with WES. QRICH2 is important for sperm motility. The mutation of QRICH2 gene, especially high frequency mutations of SMC_N domain are likely responsible for the phenotypes of both oligo-astheno-teratozoospermia and asthenospermia.