to semen analysis to confirm poor semen quality or as a possible independent diagnostic tool for assessing infertility. Overall, ORP is a reliable method of measuring OS and can be used by laboratories worldwide as a standard part of assessing semen quality.

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MULTI-CENTER EVALUATION OF OXIDATION REDUCTION POTENTIAL ASSAY IN THE INFERTILE MALE.

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OBJECTIVE: Evaluate if oxidation reduction potential (ORP) levels using the MiOXSYS analyzer could differentiate semen samples that meet the normal reference range of WHO criteria from those that do not using samples from multiple centers.

DESIGN: This study was carried out at 8 different institutions across the world. The study was approved by the Ethics committee of each participating institution (Cleveland Clinic, Cleveland, USA; Hamad Medical Center, Doha, Qatar; Dokkyo University, Osaka, Japan; The Doctor's Laboratory, London, UK; VKF American Hospital, Istanbul, Turkey; Sohag University, Sohag, Egypt; Bezmi Alem Vakif University, Istanbul, Turkey; Assam University, Silchar, India; Tulane Medical Center, New Orleans, USA) and all subjects consented prior to participation. Patients (n = 2010) were grouped into those that had all normal semen parameters (concentration, total motility, and morphology) according to WHO 2010 guidelines and those who failed to meet one or more criteria.

MATERIALS AND METHODS: Exclusion criteria included azoospermia, presence of STD or chronic disease, use of prescription, OTC medications or antioxidants. Semen parameters were assessed using the WHO fifth edition guidelines (2010). ORP was measured (mV) using the MiOXSYS system and normalized to concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration >0.999x10^6 sperm/mL were included.

RESULTS: 1804 samples had at least one abnormal sperm parameters according to WHO criteria; 206 samples had sperm parameters that fell within the normal range. ORP results were negatively correlated with sperm concentration ($p<0.01$), total sperm ($p<0.01$) progressive motility ($p<0.01$, total motility ($p<0.01$, morphology ($p<0.01$). The area under the curve (AUC) was 0.757. An ORP cut-off value of 1.34 mV/10^6 sperm/mL was able to differentiate samples with abnormal semen parameters with 58% sensitivity, 85% specificity, with positive predictive value of 96 and a negative predictive value of 42.

CONCLUSIONS: ORP levels can serve as an adjunct to routine semen analysis. Abnormal ORP levels will be especially useful in pinpointing the altered functional status of the sperm in patients with idiopathic male infertility and thereby directing those men to accurate therapeutic management.

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HIGH SEMINAL OXIDATION REDUCTION POTENTIAL IN CRYOPRESERVED SEMEN FROM INFERTILE MEN IS A MARKER OF POOR POST-THAW SPERM QUALITY.

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OBJECTIVE: Cryopreservation causes deleterious effects on human spermatozoa due to freezing and thawing, leading to decreased cryosurvival rates (CSR). The objectives of this study were to assess levels of oxidation-reduction potential (ORP) in cryopreserved semen of infertile men, and to determine their relationship to post-thaw sperm parameters.

DESIGN: A prospective cohort study.

MATERIALS AND METHODS: The study included 28 semen samples obtained from men who were evaluated for an infertility problem between November, 2016 and April, 2017. Standard semen analysis was performed according to the WHO guidelines (fifth edition, 2010). Fresh seminal ORP was measured using the MiOXSYS system (Aytu BioScience, Inc., Englewood, CO, USA). Recorded ORP values were adjusted for sperm concentration and final results were expressed as mv/10^6 sperm/ml. Semen samples with azoospermia, sperm concentration < 1 million/ml or leukocytospermia were excluded. Aliquots of 0.5 ml semen were cryopreserved using slow freezing technique. One week later, frozen samples were thawed at 37 °C, and examined for post-thaw percent of total motility, percent of progressive motility, total motile sperm (TMS) counts and ORP levels. Cryosurvival rate was calculated according to the equation: CSR = post-thaw TMS/pre-freeze TMS X 100. Data were presented as median (25^{th} and 75^{th} percentiles). Paired sample *t* test was used for comparison of the pre and post-thaw results. P value < 0.05 was considered significant.

RESULTS: Post-thaw percent of total motility [20 (10, 40)], percent of progressive motility [10 (5, 25)] and TMS counts [4.1 (0.6, 6.3) X10^6 sperm] were significantly lower than pre-freeze values (percent of total motility [50 (40, 55)], percent of progressive motility [30 (24, 35)] and TMS counts [25 (18, 41) X10^6 sperm); P values < 0.001. Post-thaw levels of seminal ORP [2.8 (2.3, 4.4) mv/10^6 sperm/ml] were significantly higher than pre-freeze values [0.9 (0.54, 1.34) mv/10^6 sperm/ml]; P < 0.001. The median percentage of CSR was 10 (5 & 20). A significant ($P< 0.05$) negative correlation was found between post-thaw levels of seminal ORP and total motility (r = -0.5), progressive motility (r = -0.41), TMS counts (r = -0.60) and CSR (r = -0.52).

CONCLUSIONS: Sperm cryopreservation in infertile men was associated with high seminal ORP, low sperm motility and reduced CSR. Sperm cryodamage is related to high seminal ORP generated during freeze-thaw process. Future efforts should be directed towards reduction in oxidant production to improve sperm recovery following cryopreservation.

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DIFFERENTIAL EXPRESSION AND LOCALIZATION OF ACE AND MAP3K3 IN OXIDATIVE STRESS RELATED MALE INFERTILITY.

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OBJECTIVE: Our earlier proteomic research on oxidative stress in infertile men demonstrated 10 differentially expressed proteins involved in three different cellular networks. These proteins [especially angiotensin-converting enzyme (ACE) and mitogen-activated protein kinase (MAP3K3)] are localized either in the center of the network or function as interlink protein between the networks. Testicular isoform of ACE is a ~80kDa protein located in the peri-acrosomal region of spermatozoa and is involved in capacitation and acrosomal activity. MAP3K3 is one of the interlink proteins that regulates flagellar movement and hyperactivation during capacitation and acrosome reaction.The objective of our study was to validate whether these two proteins can be potential biomarkers in spermatozoa under oxidative stress characterized by high levels of reactive oxygen species (ROS).

DESIGN: Validation of these potential protein biomarkers in spermatozoa of fertile donors and infertile patients during oxidative stress by Western blot (WB) analysis and immunocytochemistry.

MATERIALS AND METHODS: ROS was measured by chemiluminescence assay using luminol as probe. Both, control with normal semen parameters and infertile men were grouped into ROS (-) (<102 RLU/sec/10^6 sperm) and ROS (+) (≥102 RLU/sec/10^6 sperm). We selected 2 proteins that were either related to altered biological process or molecular function as determined in the bioinformatics analysis later on validated by WB and immunocytochemistry analysis. Protein quantification was performed by measuring relative intensity of each band and calculated using the Image J software. Subcellular protein localization was demonstrated by immunocytochemistry combining with confocal microscopy imaging.

RESULTS: WB analysis showed a 1.49 fold decrease in tACE protein in ROS (-) patients compared to ROS (-) donor (control) whereas a 2.0 fold decrease observed in ROS (+) patient group in comparison to ROS (+) donor (control) sample. In contrast, MAP3K3 was 3.14 fold increased in ROS (-) patient when compared to ROS (-) control and there was 2.01 fold increase in ROS (+) patient in comparison to ROS (+) control group. The localization of ACE was observed in the acrosome region while MAP3K3 protein localization was confirmed in the tail region.

CONCLUSIONS: We have demonstrated that infertile men with high ROS show differential expression of tACE and MAPK3 proteins reflecting these proteins as potential markers of ROS induced changes in human spermatozoa.