**Oxidative Stress**

**P-550** Wednesday, November 1, 2017

**RELATIONSHIP BETWEEN SEMINAL OXIDATION REDUCTION POTENTIAL AND SPERM DNA FRAGMENTATION IN INFERTILE MEN. A. Agarwal, M. M. Arafa, H. Elbardisi, A. Majzoub, S. S. Alsaid, E. S. Sabanegh, A. Agarwal, M. M. Arafa, R. Chandrakumar, A. Majzoub, H. Elbardisi, American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH; Urology, Hamad Medical Corporation, Doha, Qatar.

**OBJECTIVE:** Oxidation reduction potential (ORP) is a new tool to measure oxidative stress (OS) and has been shown to serve as an accurate predictor of poor semen quality in infertile men. Recent studies have shown that ORP test is a simple, quick, inexpensive and reproducible test of OS status in semen. Assessment of sperm DNA fragmentation (SDF) has been shown recently to correlate with fertility outcome in spontaneous pregnancy and assisted reproduction. OS is known as a major cause of SDF. We therefore, set out to investigate the relationship between the ORP and SDF in patients with male infertility.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Study included 399 infertile patients (Group 1) and 47 normal fertile donors (controls; Group 2) between Jan to Jun 2016 at a tertiary medical center. Patients with azospermia, leukocytospermia, history of smoking, sexually transmitted diseases or those receiving antioxidants were excluded. Data on medical history, physical examination, semen analysis, ORP and SDF testing was collected.

**RESULTS:** The mean age of all subjects was 35.6 ± 7.85, and there were no differences between the two groups. Patients had significantly higher ORP (2.74 ± 3.92 vs. 1.26 ± 1.12 mV/10^6 sperm/mL; P < 0.001) and SDF (27.6 ± 17.8% vs. 15.6% ± 6.31%, P < 0.001) than controls. ORP levels correlated significantly with SDF in all subjects (r = 0.256; P < 0.001) and in patients group (r = 0.222; P < 0.001).

**CONCLUSIONS:** Correlation of ORP levels with SDF confirms the causal relationship between OS and SDF. ORP could be used as a surrogate marker for SDF in clinics which lack access to highly complex sperm function testing due to the expense or need for highly trained laboratory personnel.

**References:**

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**OXIDATION REDUCTION POTENTIAL: A RELIABLE AND REPRODUCIBLE METHOD. A. Agarwal, M. M. Arafa, R. Chandrakumar, A. Majzoub, H. Elbardisi, American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH; Urology, Hamad Medical Corporation, Doha, Qatar.

**OBJECTIVE:** Seminal oxidative stress (OS) is well known to affect male fertility status. The lack of reproducibility in OS measurement has hindered its clinical use as a quality indicator for semen. Some tests measure single markers of oxidants or reductants, leading to lack of standardization of results. Oxidation reduction potential (ORP) can better measure OS as it provides an overall measure of the activity of both oxidants and reductants. The goals of this multicenter study was to investigate 1) the reproducibility and reliability of the ORP measurement as an indicator for sperm quality across different fertility centers and 2) establish the ORP cutoff value to distinguish infertile men from healthy controls.

**DESIGN:** ORP measurement in infertile men with abnormal sperm parameters and healthy control in two fertility centers.

**MATERIALS AND METHODS:** Semen analysis and ORP measurements from two andrology laboratories in the USA and Qatar over a period of 12 months was collected. The USA dataset contained 194 patients and 51 fertile donors, while the Qatar dataset contained 400 patients and 50 fertile donors. Semen analyses were performed followed by ORP measurements using the MiOXSYS analyzer. Semen samples with abnormal sperm parameters were identified based on the WHO 5th edition guidelines. The ORP and abnormal sperm parameters were compared using Student’s t-test and a P value of < 0.05 was considered significant. The area under the curve for different diagnostic predictive values of ORP in these groups were calculated with receiver operating characteristic (ROC) analysis.

**RESULTS:** In the USA, Qatar, and combined datasets, the infertile group had significantly lower sperm concentration, total and progressive motility, and normal morphology as well as higher ORP levels compared to fertile men (P < 0.05). When comparing data from both centers, the infertile group showed significant difference between both datasets regarding progressive motility and morphology (P < 0.001). The percentage of patients with abnormal semen volume, sperm count, total and progressive motility were significantly different between the two male infertility centers (P < 0.05). ORP levels showed no significant differences between both data sets (P < 0.08). ROC analysis indicated that ORP cut-off value of 1.42 mV/10^6 sperm can accurately differentiate fertile from infertile semen groups.

**CONCLUSIONS:** The measurements for ORP among infertile men were consistent between centers in the USA and Qatar and with previous studies conducted by our group. ORP remains stable even with measurable differences in other sperm parameters, and it therefore can be used in addition to detect at least 1 abnormal sperm parameter (70.4% sensitivity, 88.1% specificity and 95.5% positive predictive value).

**CONCLUSIONS:** ORP levels can accurately predict semen samples with poor sperm quality in infertile men. The inclusion of ORP along with routine semen analysis for male infertility evaluation will offer clinicians a more reliable method to assess sperm quality.

<table>
<thead>
<tr>
<th>ORP value in abnormal sperm parameter</th>
<th>ORP value in normal sperm parameter</th>
<th>P-value</th>
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<tbody>
<tr>
<td>11.55 (4.80, 31.34) (n=92, oligozoospermia)</td>
<td>0.94 (0.43, 1.56) (n=102, normal concentration)</td>
<td>&lt;0.001</td>
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<tr>
<td>6.91 (1.58, 20.08) (n=102, asthenozoospermia)</td>
<td>1.27 (0.44, 2.76) (n=91, normal motility)</td>
<td>&lt;0.001</td>
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<tr>
<td>2.42 (1.06, 10.28) (n=95, teratozoospermia)</td>
<td>1.20 (0.50, 3.81) (n=79, normal morphology)</td>
<td>=0.003</td>
</tr>
<tr>
<td>3.76 (1.19, 16.40) (n=152, at least 1 abnormal sperm parameter)</td>
<td>0.74 (0.41, 1.30) (n=42, normal sperm parameters)</td>
<td>&lt;0.001</td>
</tr>
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</table>

Values are median (25th, 75th percentile).
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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MULTICENTER EVALUATION OF OXIDATION REDUCTION POTENTIAL ASSAY IN THE INFERTILE MALE. A. Agarwal, R. Chandrakumar, M. M. Arafa, H. Elbardisi, H. Okada, K. Suzuki, S. Homa, A. Killeen, B. Balaban, A. Ayaz, R. Saleh, A. Armagan, S. Roychoudhury, S. C. Sikka. American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH; Urology, Hamad Medical Corporation, Doha, Qatar; Urology, Dokkyo Medical University, Koshigaya, Japan;* The Doctor’s Laboratory, London, United Kingdom; †American Hospital of Istanbul, Turkey; ‡Urology, Sohag University, Sohag, Egypt; §Urology, Bezmi Alem Vakıf University, Istanbul, Turkey.

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; VVF American Hospital of Istanbul, Turkey; Sohag University, Sohag, Egypt; Bezmi Alem Vakıf 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.

RESULTS: 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/10⁶ sperm/µl). For group comparisons, only those samples with a concentration >0.999x10⁶ sperm/µl were included.

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. R. Saleh, A. Agarwal, M. Elsity. Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag University, Sohag, Egypt; †Ayial Hospital, Sohag, Egypt; ‡Urology, Cleveland Clinic, Cleveland, OH.

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 (Ayto BioScience, Inc., Englewood, CO, USA). Recorded ORP values were adjusted for sperm concentration and final results were expressed as mV/10⁶ sperm/µl. Semen samples with azoospermia, sperm concentration < 1 million/µl 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 (25th and 75th 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) X10⁶ 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, 34) X10⁶ sperm]). P values < 0.001. Post-thaw levels of seminal ORP [2.8 (2.3, 4.4) mV/10⁶ sperm/µl] were significantly higher than pre-freeze values [0.9 (0.54, 1.34) mV/10⁶ sperm/µl; 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 cryopreservation 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. A. Ayaz, N. Kothandaraman, R. Agarwal, Z. Cakar, S. Sikka, E. S. Sabanegh, R. Sharma. †American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH; °Urology, Tulane University Health Science Center, New Orleans, LA; ‡Urology, Cleveland Clinic, Cleveland, OH.

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 isofrom of ACE is a ~80kDa protein located in the peri-acrosomal region of spermatozoa and is involved in capacitation. Alysosomal acrosomal protein 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 immunochemistry.

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/10⁶ sperm) and ROS (−) (≥102 RLU/sec/10⁶ 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 in comparison to ROS (+) control group. 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.

CONCLUSIONS: We have demonstrated that infertile men with high ROS show differential expression of tACE and MAP3K3 proteins reflecting these proteins as potential markers of ROS induced changes in human spermatozoa.