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2015-2016
ASRM 2015
Effect of time on ORP in semen and seminal plasma

ASA 2016
ORP is related to abnormal semen parameters in male factor infertility
Measurement of ORP as a newer tool indicative of OS in infertile men with leukocytospermia

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Validation of ORP in fresh/frozen samples with MiOXSYS®

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ORP predictor for sperm morphology in infertile men
Seminal fluid static ORP is lower in seminal fluid that meets all who criteria for fertility
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ASRM 2016
ORP a novel test for evaluating male infertility
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Infertile men have a redox imbalance that distinguishes them from fertile men
ORP can differentiate fertile from infertile men
Evaluation of intra and inter observer reliability
Establishing the Oxidation-Reduction Potential in Semen and Seminal Plasma

Ashok Agarwal, PhD1, Stefan S. Du Plessis, PhD1,2, Rakesh Sharma, PhD1, Luna Samanta, PhD1,3, Avraham Harlev, MD1,4
Gulfam Ahmad, PhD1,3, Saaj Gupta, MD1,4 and Edmund S. Sabanegh, MD1

1American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH; 2Medical Physiology, Stellenbosch University, Tygerberg, South Africa; 3Redox Biology Laboratory, School of Life Science, Ravenna University, Orosia, India; 4Soroka Medical Center, Ben-Gurion University, Beer Sheva, Israel. 2Department of Urology, Cleveland Clinic, Cleveland, OH

ABSTRACT

OBJECTIVE: Oxidation-reduction potential (ORP) is a novel measure of oxidative stress or redox imbalance in biological samples. Static ORP (sORP) provides an integrated measure of the balance between total oxidants and reductants in a biological system, whereas capacity ORP (cORP) equals the amount of antioxidant reserves. ORP has been shown to be correlated with illness and injury severity that accompanies the state of oxidative stress; cORP correlates with the ability to respond to illness or injury. Our objectives were to evaluate whether cORP could be measured in semen and seminal plasma samples, and whether cORP levels correlate with seminal plasma ORP levels. DESIGN: Prospective study measuring ORP in both semen and seminal plasma. MATERIALS AND METHODS: Seventy-seven male volunteers with normal semen analysis were recruited (36 Semen, 41 Seminal plasma). Semen and seminal plasma samples were measured for sORP (mV/106 sperm) and cORP (µC/106 sperm). A ROC analysis was used to determine the preliminary predictive value of sORP levels in semen samples. RESULTS: cORP levels in semen correlated significantly with the values in seminal plasma. Correlation coefficient (r) between sORP in semen and seminal plasma (r=0.609; P=0.004) and cORP (in microcoulombs or µC) were recorded. Analysis was performed in triplicate and the average values were converted for sperm concentrations and expressed as sORP (mV/106 sperm) and µC/106 sperm. CONCLUSIONS: cORP accurately measured in semen and seminal plasma. Based on high sensitivity as assessed by ROC analysis, the sORP levels can be used to screen infertile men with oxidative stress. These results are being validated in a larger cohort of infertile men.

RESULTS: After obtaining, Institutional Review Board approval, a prospective study was carried out on 18 normal healthy men. All subjects provided written consent to be enrolled in this study. After liquefaction, a standard semen analysis was performed. Semen samples were subsequently divided into two fractions and the seminal plasma was isolated from one fraction (300g, 7min) before measuring the sORP and cORP in both fractions with the MiOXSYS system (Aytu BioSciences). Spearman correlation test was used for statistical analysis to compare qualitative variables. A P value of < 0.05 was considered statistically significant. A Receiver Operating Characteristic curve (ROC) was used to determine the predictive discriminatory power of sORP values to identify semen samples with poor motility.

INTRODUCTION

Male factor infertility is a significant medical condition accounting for up to half of all cases of couple infertility. It affects approximately one in 20 men in the general population and no identifiable cause can be found in over 25% of infertile males. Oxidative stress is well described as a leading contributing factor to male infertility and evidence now suggests that reactive oxygen species (ROS)-mediated damage to sperm is a significant contributing pathology in 30-50% of cases. The quality of semen is considered a key indicator in male fertility, yet a significant proportion of male infertility remains unexplained in part because of the lack of standardized tests available to clinicians and researchers to assess oxidative stress.

Currently, the most common methods for measuring oxidative stress is by measurement of the levels of ROS by chemiluminescence assay in semen and total antioxidant capacity (TAC) by colorimetric assay in the seminal plasma and subsequently calculating a composite ROS/TAC score. However, the current methods used to measure oxidative stress (use of sophisticated instruments, are cumbersome, require larger sample volume, and increase the turn-around time for these tests). Redox imbalance is caused by a higher production of ROS and reactive nitrogen species or a decrease in endogenous antioxidant defenses. Semen samples have historically been used to measure redox imbalance in patients (e.g., antioxidants like glutathione, lipid peroxidation, free radical production, protein oxidation, and/or enzyme activity). Relying upon measurements of individual markers often results in an unreliable, variable, and conflicting measurement of a patient’s redox balance. Oxidation-reduction potential (ORP) is a measure of oxidative stress or redox imbalance in biological samples. Static ORP (sORP) provides an integrated measure of the balance between total oxidants and reductants in a biological system, whereas capacity ORP (cORP) equals the amount of antioxidant reserves. ORP has been shown to be correlated with illness and injury that accompanies the state of oxidative stress; cORP correlates with the ability to respond to illness or injury.

RESULTS

1. Sensory-ORP levels (3.30±3.85 mV/106 sperm) correlated significantly with the sORP levels in seminal plasma (3.21±3.65 mV/106 sperm) (Figure 2A).

2. Sensory-ORP levels (0.92±2.955 µC/106 sperm) correlated significantly with the cORP levels in seminal plasma (1.09±3.192 µC/106 sperm) (Figure 2B).

3. A significant negative correlation existed between sperm mobility and cORP in semen (n=0.609; P=0.004) (Figure 2C).

4. A significant negative correlation existed between sperm motility and sORP in seminal plasma (n=0.698; P=0.002) (Figure 2D).

5. For improved semen analysis, a high level of sperm in semen (sensitivity = 100%, specificity = 89.3%, AUC=0.947) and 4.56mV/106 sperm in seminal plasma (sensitivity = 100%, specificity = 93.8%, AUC = 0.969) was highly predictive of abnormal sperm motility, i.e. <40% total motility (Figures 3 and 4).

CONCLUSIONS

1. The MiOXSYS test can accurately measure ORP in semen and seminal plasma.

2. Both semen and seminal plasma can be used to measure sORP and cORP levels without differing significantly.

3. Higher sORP measures are associated with lower levels of sperm motility.

4. Based on high sensitivity, as assessed by ROC analysis, the sORP levels might be used to screen infertile men with mobility abnormalities.

MATERIALS and METHODS

After liquefaction, standard semen analysis was performed according to WHO 2010 guidelines to determine sperm concentration and motility. Semen was stained with a Diff-Quik kit for assessment of sperm morphometry. Sample was tested for Leukocytospermia, i.e. >1 X 106 WBC/mL when the round cell concentration was >1 X 106 and confirmed by the peroxidase or the Endtz test.

Oxidation-Reduction (ORP) Measurement

ORP was measured using the MiOXSYS comprised of a RedoxSYS analyzer (Figure 1) and MiOXSYS sensor (Figure 2). The sensor was inserted face-up and with the sensor electrodes facing the MiOXSYS Analyzer. Using a micropipette, 30µL of sample was expressed as sORP (mV/106 sperm) and µC/106 sperm.

Semen Analysis

Semen samples with a sperm concentration higher than 1 X 106 sperm/mL were not used for the analysis. A Cutoff = 4.65sORP/106 sperm in seminal plasma was considered in sperm samples with poor motility.

Statistical Analysis

Spearman correlation test was used for statistical analysis to compare qualitative variables. A p value of < 0.05 was considered statistically significant. A Receiver Operating Characteristic curve (ROC) was used to determine the predictive discriminatory power of sORP values to identify semen samples with poor motility.

ORP Levels in Semen and Seminal Plasma

Semen cORP (µC/106 sperm)

ORP Levels in Semen and Seminal Plasma

Semen cORP (µC/106 sperm)

Seminal Plasma cORP

Semen cORP (µC/106 sperm)

Figure 1

Figure 2

Figure 3

Figure 4
Semen Oxidative Reduction Potential (ORP) is related to Abnormal Semen Parameters in Male Factor Infertility

Department of Urology, School of Medicine, Tulane University, 1430 Tulane Avenue, New Orleans, LA-70112.

Introduction
Oxidative stress (OS) is known to impair the physiological function of spermatozoa leading to male infertility. OS occurs due to imbalance resulting from higher production of reactive oxygen species (ROS) and lower activity of the antioxidant enzymes in semen. The assessment of such imbalance is complicated and often difficult to investigate in the clinical setting. Oxidation-reduction potential (ORP) is a newer and simpler tool to assess the status of oxidative stress. The present study measures both static-ORP (sORP as mVolt) and capacity-ORP (cORP current as μA) in fresh semen samples using a new electrochemical system, MIOXSYS™. To correlate ORP (as a measure of oxidative stress) with semen parameters of infertile men.

Objectives
To understand the potential impact of sORP and cORP in semen of infertile men, (a) who have shown improvement in their semen parameters over time, and (b) who have consistently low semen parameters after follow-ups.

Materials and Methods
Semen samples from twenty four infertile men attending our andrology clinic were collected after informed consent. Semen analyses were first performed according to the WHO guidelines. A well-mixed aliquot of 30μl was then used to measure sORP and cORP using the MIOXSYS analyzer after calibration. All values reported are mean ± SEM and the Spearman correlation coefficient (r) carried out using GraphPad Prism software.

Results (Continued)
Figure 2: Shows sORP/million sperm/ml levels in whole semen of infertile men and its correlation with sperm concentration, total sperm count, sperm motility and morphology. 2a: sORP levels were measured in fresh whole semen from infertile men with improved/normal sperm concentration (NC), low sperm concentration (LC), improved/normal total sperm count (NTC), low total sperm count (LTC), improved/normal sperm motility (NM), and low motility (LM) using the new electrochemical system, MIOXSYS™. Infertile bar shows composite data of all groups taken together. All data are expressed as mean±SEM values. Significant differences are represented as the p-value <0.05 (*). 2b: Scatter plot depicting significant negative correlation between sORP in fresh whole semen from infertile males and sORP/million sperm/ml levels (one data point is outside the axis limits). The solid line represents the linear regression of points and dotted line represents 95% confidence interval for the mean values. Rho- and p-values are for the Spearman’s rank correlation test.

Conclusions
1. sORP in the semen samples of infertile men correlated well with their sperm concentration, motility, and morphology.
2. A significantly high sORP/cORP ratio as assessed by MIOXSYS™ in infertile patients with low sperm concentration and motility suggests the role of increased oxidative stress.
3. This newer system (MIOXSYS™) measuring ORP could potentially serve as an important additional indicator in the workup of male factor infertility.
4. These results provide evidence that sORP and cORP levels in semen of infertile men can differentiate and predict responders vs non-responders to infertility therapy.

Figure 3: Shows cORP levels (µC) and sORP/cORP ratio in whole semen of infertile men with normal (improved) and low semen parameters over time, as well as its negative correlation with sORP.

3a: cORP levels were measured in fresh whole semen from infertile men with improved/normal sperm concentration (NC), low sperm concentration (LC), improved/normal total sperm count (NTC), low total sperm count (LTC), improved/normal sperm motility (NM), and low motility (LM) using the new electrochemical system, MIOXSYS™. Infertile bar shows composite data of all groups taken together. All data are expressed as mean±SEM values. Significant differences are represented as the p-value <0.05 (*). 3b: Scatter plot depicting significant negative correlation between cORP in fresh whole semen from infertile males and sORP/million sperm/ml levels (one data point is outside the axis limits). The solid line represents the linear regression of points and dotted line represents 95% confidence interval for the mean values. Rho- and p-values are for the Spearman’s rank correlation test.
Measurement of Oxidation-Reduction Potential (ORP) as a new tool indicative of Oxidative Stress in Infertile Men with Leukocytospermia

Suresh C. Sikka, Jaideep S. Toor, Lucelia Daniels, Faysal A. Yafi, and Wayne J.G. Hellstrom.
Department of Urology, Tulane University, New Orleans, Louisiana.

Introduction

- Leukocytospermia (LCS, a common cause of male infertility) is characterized by the presence of >1X10^6/mL white blood cells (WBC) in the semen sample.
- LCS caused by prostatitis and genitourinary inflammation (GUI), manifested by inflammatory chemokines and increased oxidative stress (OS) in the semen results in decreased sperm motility, high sperm DNA damage, decreased sperm function, i.e., fertility.
- Oxidation-reduction potential (ORP) is a newer integrated measure of the balance between total oxidants (ROS) and reductants (antioxidants) that reflects oxidative stress status (OSS) in a biological system.
- However, the current methods to measure OSS are time consuming, expensive, need fresh sample, have many limitations and thus are not routinely performed.
- This preliminary study measures both static-ORP (sORP as mVolt) and capacity-ORP (cORP as µC) in fresh semen samples using the new electrochemical system, MiOXSYS™.

Materials and Methods

- Semen samples from LCS and non-LCS infertility patients in our clinic were collected, evaluated for semen parameters, and processed for:
  (a) Expression/localization of TLR-4 and COX-2 in washed sperm by immunofluorescence microscopy. "Image J" software (NIH) was used to quantify fluorescence intensity.
  (b) 30µl aliquot was placed on the special sensor in the calibrated MiOXSYS Sensors.

Results

- Our previous and current studies showed TLR-4 translocation from cytoplasm to nucleus and overexpression of COX-2, indicative of active inflammation in parallel with high WBC content during leukocytospermia.
- Both TLR-4 and COX-2 are potential biomarkers for GUI and male factor infertility.
- MiOXSYS System ORP data suggest higher oxidation-reduction potential and one-step indicator of increased oxidative stress in semen samples of infertile men with leukocytospermia.
- This newer ORP parameter as measured by MiOXSYS could potentially serve as an important additional indicator in the workup of male factor infertility. Much larger standardized studies in a multicenter format are needed to establish such diagnostic role of MiOXSYS in evaluation of OSS in routine clinical practice.

Conclusions

- All values were normalized to sperm concentration and expressed as Mean ± SEM units/million sperm/mL.

- MiOXSYS analyzer to measure sORP and cORP.
Validation of oxidation-reduction potential in fresh and frozen semen samples with MiOXSYS® System

Ashok Agarwala*, Rakesh Sharma, Stefan Du Plessis and Edmund Sabanegh
American Center for Reproductive Medicine and Department of Urology, Cleveland Clinic, Cleveland, OH

ABSTRACT

INTRODUCTION: Oxidative stress in seminal ejaculate is quantified by measuring individual markers such as reactive oxygen species, lipid peroxidation and total antioxidants. However, they have limited diagnostic capabilities. ORP is a measure of oxidative stress that is a balance between total oxidants (RO) and total reductants (AHR). ORP can be measured in fresh and frozen semen following exogenous induction of oxidative stress using the analyzer.

MATERIALS AND METHODS: ORP was measured by MiOXSYS System in both fresh and frozen semen samples. The change from pre- to post-frozen sample was used to compare. The MiOXSYS System was calibrated according to the manufacturer’s instructions. Table 1 shows the comparison of oxidation-reduction potential (ORP) following exposure to 50 µm (P<0.001) in both pre- and post-freeze semen samples. Samples that were highly viscous or had >1x106/mL round cells were excluded from the study.

RESULTS: The MiOXSYS System can measure the ORP in both fresh and frozen semen samples. It is simple and real-time measure of oxidative stress that can be used for fertility laboratories.

CONCLUSIONS: MiOXSYS® System can measure ORP in both fresh and frozen semen samples in real-time measure of oxidative stress and ORP in Andrology and IVF laboratories. Further studies utilizing larger sample size and in multicenter format using semen samples from healthy men are important to verify its clinical utility as an alternative to current oxidative stress markers. This will introduce an easy, quick, and real-time measure of oxidative stress and ORP in Andrology and IVF laboratories.

Figure 1. Measurement of oxidation-reduction potential (ORP) by: A: Analyzer by placing the sensor; B: gently rinsing the sensor; C: view the sensor when it is in place.

Table 1. Pre- and post-thaw seminal sORP and cORP after exposure to cumene hydroperoxide

Table 2. Effect of freezing on seminal sORP and cORP after exposure to cumene hydroperoxide

CONCLUSIONS

1. MiOXSYS® System can measure the ORP in both fresh and frozen semen samples in real-time measure of oxidative stress and ORP in Andrology and IVF laboratories.

2. ORP technique is sensitive to changes in ORP following induction of oxidative stress by cumene hydroperoxide.

3. Induction of oxidative stress by CH and measurement of ORP in real time supports the diagnosis of the MiOXSYS® System as evaluating ORP and cORP and validates the measurements in humans.

4. Further studies utilizing larger sample size and in multicenter format using semen samples from healthy men are important to verify its clinical utility as an alternative to current oxidative stress markers. This will introduce an easy, quick, and real-time measure of oxidative stress and ORP in Andrology and IVF laboratories.
Semen Fluid Static Oxidation-Reduction Potential is Lower in Seminal Fluid that Meets All WHO Criteria for Fertile Men

El-Bardisi, M1, Arvad, M1, Agarwal, A1, Allaf, S1, Majzoub, A1,2, Keyserthilt, F1, Al-Rumaihi, K1, Bar-Or, D5, Bjugstad, K5
1. Urology Department - HMC - Doha, Qatar, 2. Andrology Department, Cairo University, Egypt, 3. Center for Reproductive Medicine, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA, 4. Department of Urology, Glickman Urological and Kidney Institute, 5. Swedish Medical Center, Englewood, CO USA.

Background and Study Question
Oxidative stress is an imbalance of free radicals, oxidants activity, and oxidative damage. The seminal plasma itself can be considered a redox medium as the balance between oxidants and antioxidants is a dynamic equilibrium. Oxidative stress plays a critical role in many aspects of semen quality. For example, oxidative stress has been associated with azoospermia, a reduced sperm count, and reduced sperm motility, all of which affect fertility negatively.

Aims
To measure the ORP in semen samples of infertile men and assess its association with semen parameters and fertility outcome.

Materials and Methods
N = 248; M = 60.2; SD = 22.1

Results
Semen with normal morphology and normal semen parameters had significantly lower sORP values than those that fail one or more parameters (*p < 0.05). Abnormal morphology contributed to increasing sORP values from those that fail one or more semen parameters, including a state of oxidative stress. This implies that ORP may be used to clarify the relationship between the redox system and semen parameters associated with infertility. Further, a sORP cut-off value may identify semen samples that meet the normal reference range of WHO parameters from ones that fail one or more.

Discussion
This study suggests that measuring sORP might provide users with an independent measure of the oxidative stress in semen and thus clarify the relationship between the redox system and semen parameters associated with infertility. Further, a sORP cut-off value may identify semen samples that meet the normal reference range of WHO parameters from ones that fail one or more.

Table 1: Patient Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal (N)</th>
<th>Abnormal (N)</th>
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</thead>
<tbody>
<tr>
<td>Characteristics (%)</td>
<td>352</td>
<td>246</td>
</tr>
<tr>
<td>Sensitivity (95%)</td>
<td>60.2</td>
<td>65.9</td>
</tr>
<tr>
<td>Specificity (95%)</td>
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<td>86.6</td>
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<tr>
<td>Positive Predictive</td>
<td>79.7</td>
<td>88.6</td>
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<tr>
<td>Negative Predictive</td>
<td>17.9%</td>
<td>12.5-24.2%</td>
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<tr>
<td>Accuracy</td>
<td>67%</td>
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</table>

Table 2: ROC results based on a cut-off of 1.635 sORP to identify abnormal semen samples that fail WHO criteria

<table>
<thead>
<tr>
<th>Test</th>
<th>Data Set 1</th>
<th>Data Set 2</th>
<th>Data Set 3</th>
<th>Combined Data Set</th>
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<tbody>
<tr>
<td>NPV (%)</td>
<td>68.5</td>
<td>60.2</td>
<td>65.9</td>
<td>65%</td>
</tr>
<tr>
<td>Specificity (95%)</td>
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<td>94.1</td>
<td>93.9</td>
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<td>Sensitivity (95%)</td>
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<td>63.1</td>
<td>64.8</td>
<td>63.1</td>
</tr>
<tr>
<td>Positive Predictive</td>
<td>86.6</td>
<td>87.0</td>
<td>87.0</td>
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<tr>
<td>Negative Predictive</td>
<td>13.4%</td>
<td>12.5-24.2%</td>
<td>11.1-21.9%</td>
<td>12.5-24.2%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>77%</td>
<td>78%</td>
<td>77%</td>
<td>77%</td>
</tr>
</tbody>
</table>

Figure 1: Those that met WHO criteria for normal sperm have significantly lower sORP values than those that fail one or more parameters (*p < 0.05).

Figure 2: Semen with abnormal morphology and normal semen parameters have significantly higher sORP values (*p < 0.05).

Figure 3: Abnormal progressive motility alone does not elevate sORP values, but the addition of abnormal morphology indicates a significant elevation. Progressive motility (97.3% of samples) was the most common abnormal parameter followed by morphology (79.7% of samples; *p < 0.05).

Semen with abnormal parameters have higher levels of oxidative stress. By measuring sORP, a cut-off value of 3.29mV/sperm concentration was able to reliably predict abnormal morphology.

This study suggests that measuring sORP might provide users with an independent measure for confirming semen morphology.

Table 3. sORP cut-off value to identify abnormal morphology (mV/mL risk of 95% CI)

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Sensitivity (95%)</th>
<th>Specificity (95%)</th>
<th>Positive Predictive</th>
<th>Negative Predictive</th>
<th>Accuracy</th>
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<tbody>
<tr>
<td>1</td>
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</tr>
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<td>2</td>
<td>62.5</td>
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<tr>
<td>3</td>
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<td>93.9</td>
<td>11.1-21.9%</td>
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</tr>
<tr>
<td>Combined</td>
<td>63.1</td>
<td>87.0</td>
<td>93.9</td>
<td>12.5-24.2%</td>
<td>77%</td>
</tr>
</tbody>
</table>
Introduction
Oxidation-reduction potential (ORP) of spermatozoa Selected
for Intracytoplasmic Sperm Injection (ICSI) After Exposure to Polyvinylpyrrolidone (PVP) and Hyaluronic Acid (HA)
Ashit Agarwala, Shubhadeep Roychoudhury, Sandro Esteves,
Israel Maldonado Rosado, Rakesh Sharma, and Sajal Gupta

Abstract
We assessed the ORP of human spermatozoa after exposure to PVP or HA prior to ICSI. The mean 
ORP (mV/106 sperm/mL) in the control group was 57.60 ± 0.63. After 20 minutes of exposure,
the ORP level in the control group was 47.04 ± 1.01 mV/106 sperm/mL, 50.80 ± 1.23 mV/106 sperm/mL
and 50.88 ± 1.38 mV/106 sperm/mL for PVP, HA and control groups, respectively. Statistical significance 
was set at a p value of p<0.05.

Results
The ORP of human spermatozoa selected for ICSI after exposure to PVP or HA was significantly different 
from the control group (p<0.05). The ORP level was lowest in the PVP-treated group, possibly because 
PVP contains a synthetic serum replacement--a chelating agent not present in HA. The findings indicate 
that PVP may have antioxidant properties and support continued use of PVP for sperm manipulation in 
ICSI procedures.

Materials and Methods
Healthy semen samples from normozoospermic men with normal semen parameters were used as controls. 
Sperm preparations were adjusted to 5x106 sperm/mL. A sperm aliquot was then incubated with either 
PVP or HA. Static ORP (sORP) was measured using a novel galvanostat-based technology--the MiOXSYS System 
(Aytu Biomedical, Englewood, CO). The ORP was measured at room temperature. Data were collected at 
represented as mV/106 sperm/mL and analyzed for statistical significance at the level of P<0.05.

Conclusions
The ORP of human spermatozoa selected for ICSI after exposure to PVP or HA was significantly different 
from the control group (p<0.05). The ORP level was lowest in the PVP-treated group, possibly because 
PVP contains a synthetic serum replacement--a chelating agent not present in HA. The findings indicate 
that PVP may have antioxidant properties and support continued use of PVP for sperm manipulation in 
ICSI procedures.
INTRODUCTION

Male infertility is a relatively common medical condition affecting up to 12% of men globally. Sperm parameters are poor surrogate measures of a man's ability to father. Advanced tests of sperm function have been proposed as alternative methods that can enhance the diagnostic accuracy of male infertility, particularly in cases of unexplained infertility, one or more abnormal semen parameters, recurrent pregnancy loss, or failure of intracytoplasmic sperm injection. Electrochemical detection of oxidative stress (OS) resulting from a number of endogenous and exogenous causes is believed to play a central role in the pathogenesis of male infertility. Measurement of ORP (oxidation-reduction potential) has been used as a novel method for oxidative stress testing. Results distinguished between healthy normal semen and male factor infertility patients. These findings suggest that ORP is an important parameter that plays a significant role in male infertility, and thus its assessment and management are critical for patient care.

Currently available assays for OS measure only certain or a discrete quantity of oxidants (RSS; by chromametric assay), antioxidants (TTC assay) or post-harvest damage (HARA). Such tests are also tedious, time consuming, and require special technical skills and large sample volumes. As a result, a new method for the rapid and accurate measurement of ORP in semen is necessary. For ORP measurement, the MiOXSYS system was used, which is a rapid and accurate method for measuring ORP in semen.

MATERIALS AND METHODS

The objective of our study was to establish a reference value for ORP in semen to distinguish between normal semen and abnormal semen (at least one semen parameter <15 x10^6 sperm/mL; at least one sperm motility <30%; at least one sperm concentration <1 X10^6 sperm/mL; at least one sperm morphology <4%; or at least one round cell concentration >4% in a semen sample). A cutoff value of 1.36 mV/10^6 sperm/mL can serve as a preferred cutoff value for distinguishing the prevalence of OS in male factor infertility.

The study population was divided according to the results of the semen analyses into a normal semen group and a normal semen group. The normal semen group had semen volume >1.5 mL, sperm concentration >15 x10^6 sperm/mL, total sperm count >39 x10^6 sperm/mL, normal motility >60%, and normal morphology >40% in the normal semen group. These parameters historically define the WHO 2010 WHO normal reference ranges.

Background information on the study population is presented in Table 1 along with a comparison of semen parameters between the control and infertile groups. Figure 1 shows the distribution of ORP in semen samples and the normal range according to the MiOXSYS® analyzer. Box Plot distribution of ORP in semen samples and the normal range according to the MiOXSYS® analyzer is presented in Figure 3. Normal ORP (mV/10^6 sperm/mL) values were found in all samples from normal semen and in 92% of samples from infertile semen.

RESULTS

Distribution of ORP in semen samples according to the MiOXSYS® System. A. Normal healthy controls. B. Controls with proven fertility. C. Controls with unproven fertility. D. Infertile patients showing a wider range and sample point past the sample point in controls.

CONCLUSIONS

1. ORP test in semen using the MiOXSYS® System is an alternative method for measuring OS and distinguishing normal semen from male factor infertility patients.

2. Sperm parameters related to male infertility are under normal men from male factor infertility patients.

3. An ORP cutoff value of 1.26±0.56 mV/10^6 sperm/mL in semen can distinguish between normal men and patients with male factor infertility.

Oxidative stress (OS) is associated with male infertility. The measurement of oxidation-reduction potential (ORP) represents a basis for the evaluation of the redox state of the seminal plasma and seminal fluid. A. MiOXSYS® Analyzer showing the sensor module and the sensor module. B. D. MiOXSYS® herself showing the sensor module and the sensor module. C. D. MiOXSYS® herself showing the sensor module and the sensor module.

A. Normal healthy controls. B. Controls with proven fertility. C. Controls with unproven fertility. D. Infertile patients showing a wider range and sample point past the sample point in controls.

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A. Normal healthy controls. B. Controls with proven fertility. C. Controls with unproven fertility. D. Infertile patients showing a wider range and sample point past the sample point in controls.

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OXIDATION REDUCTION POTENTIAL – A NOVEL MARKER OF VARICOCELE PATHOPHYSIOLOGY

Ashok Agarwal, PhD., Ahmad Mazjoub, MD., Shubhadeep Roychoudhury, PhD., and Rakesh Sharma, PhD
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH

ABSTRACT

Objectives: Oxidative stress (OS) plays a role as a common denominator in the different etiologies of male infertility. In this study, we evaluated the oxidative stress in infertile men with clinical varicoceles to examine the role of OS as a contributing factor to male infertility.

Complex: The oxidative stress in infertile men with clinical varicoceles was evaluated by measuring the oxidative stress marker, ORP

Materials and Methods: We measured ORP and examined its correlation with OS markers in 38 infertile men presenting with primary infertility and compared them with 13 fertile controls. Semen analysis was performed after complete liquefaction at 37°C for 20 minutes.

RESULTS: The patients mean age ± SEM was 33 ±1.2 years. Compared with controls, grade 3 varicocele patients had lower sperm concentration. ORP levels were higher in all the infertile groups than in the controls. A comparative analysis of semen parameters and ORP is shown in Table 1. A significant correlation was observed between the ORP level and varicocele grade (p<0.05). Morphology was significantly higher in the control group. ORP levels were higher in all the infertile groups than in the controls.

CONCLUSIONS: Our study confirms the oxidative stress (OS) markers in infertile men with varicoceles.

INTRODUCTION

Male infertility is a relatively common medical condition affecting up to 12% of men globally. Varicocele is the most common correctable cause of male infertility. Oxidative stress is a contributor of varicocele. The World Health Organization (WHO) has acknowledged OS as an important parameter that plays a significant role in male infertility.

Individual markers of oxidative stress such as ROS measured by chemiluminescence assay, antioxidants (TMC assay) or post-harvest damage (MDA assay) are common. However such tests are also tedious, time consuming, and require special technical skills and large sample volumes. A measure that comprises all known and unknown oxidants and has no antioxidant activity in a semen sample would be an ideal marker of male infertility.

Oxidation-reduction potential (ORP) provides a comprehensive measure of the redox system and of OS. We have established the ORP levels of 1.36 mV/10^6 sperm/mL, that differentiates intertile men from healthy men. The objective of our study was to establish the ORP levels in intertile men with clinical varicocele and compare them with intertile men with idiopathic infertility.

MATERIALS and METHODS

Following institutional approval, semen samples were obtained from 15 healthy men according to the WHO 5th edition guidelines (World Health Organization, 2010). The infertile group was comprised of 51 patients presenting to the male infertility with varicocele (n=38) and 13 subjects with idiopathic infertility. The exclusion criteria for both patients and control groups with respect to smoking, alcohol and medications. All patients were included who were treated with microsurgical varicocelectomy.

Prospecive case-control pilot study.

Materials and Methods: Thirty-eight patients with clinical varicocele presenting with primary infertility were randomized to the varicocele group. Fifteen patients with idiopathic infertility and 13 fertile controls were included. Wilxcon’s rank sum test was used to compare the groups with respect to ORP evaluation. O value of 1.36 mV/10^6 sperm/mL is depicted in Figure 2.

Table 1: Comparison of semen analysis and ORP levels between study groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=38)</th>
<th>Grade 1 (n=10)</th>
<th>Grade 2 (n=10)</th>
<th>Grade 3 (n=10)</th>
<th>Infertility (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration (×10^6/mL)</td>
<td>2.82 ± 0.4</td>
<td>3.03 ± 0.4</td>
<td>3.15 ± 0.4</td>
<td>3.32 ± 0.4</td>
<td>4.01 ± 0.4</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>7.8 ± 0.7</td>
<td>3.15 ± 0.6</td>
<td>3.73 ± 0.7</td>
<td>3.69 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>2.82 ± 0.4</td>
<td>3.25 ± 0.4</td>
<td>3.15 ± 0.4</td>
<td>3.22 ± 0.3</td>
<td>4.01 ± 0.8</td>
</tr>
</tbody>
</table>

CONCLUSIONS

1. Men with clinical varicocele have semen parameters that are under a state of oxidative stress and have elevated levels of ORP.
2. ORP is a single measure of oxidative stress in semen samples that can be easily measured in both men with clinical varicocele and idiopathic infertility.

Figure 1. Measurement of oxidation reduction potential by the MiOXSYS System. A: MiOXSYS Analyzer showing the sensor socket and the sensor module and B: Sensor showing the reference cell and the sample port where the sample is loaded.

Figure 2. Distribution of ORP in: A: controls with proven fertility; B: Interfertile patients presenting with a clinical varicocele, and C: Idiopathic infertility. ORP values are presented as median and 25%, 75% percentile.
Infertile Men Have A Redox Imbalance That Distinguishes Them From Fertile Men

A. Ayaz1, K.B. Bjugstad2, D. Bar-Or3, A.Armagan4
1 Yildiz Technical University, Istanbul, Turkey 2 Aytu Bioscience, Englewood, CO 3Trauma Research, Swedish Medical Center, Englewood, CO 4 Urology, Bezmialem Vakif University, Istanbul, Turkey

OBJECTIVE

The Redox system is carefully balanced between the activities oxidant to reductants based on biological need. In fertility, a limited amount of oxidant activity is required to support sperm capacitation and embryonic stem cell differentiation. However, excessive amount of this activity causes oxidative stress which damages lipids, proteins and result in DNA fragmentation. The goal of the study was to determine if the imbalance in the redox system in infertile men was statistically significant to distinguish them from fertile men, and to identify any corresponding semen parameters that may account for this difference.

MATERIAL AND METHODS

Participants were recruited from an international fertility clinic; 34 men with diagnosed primary infertility and 47 who had fathered a child in the last 24 months. Semen samples were analyzed respecting WHO Manual 5th Edition criteria.

RESULTS

Infertile donors had higher sORP values than fertile. The odds of being infertile with a sORP value greater than 1.36 mV/106/mL was 9.76 times higher than if the sORP was lower. 63% of donors had abnormal morphology, including 38% of fertile and 97% of infertile.

<table>
<thead>
<tr>
<th># of Failed Parameters</th>
<th>N (%)</th>
<th>Most common failed parameter</th>
<th>N (%)</th>
<th>Most common failed parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19/47</td>
<td></td>
<td>1/2.9</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18/20</td>
<td>50.0% morphology</td>
<td>23/67.6</td>
<td>100% morphology</td>
</tr>
<tr>
<td>2</td>
<td>10/22</td>
<td>50.0% total and progressive</td>
<td>9/36.5</td>
<td>88.9% morphology and total numbers</td>
</tr>
<tr>
<td>3</td>
<td>0/4</td>
<td>1/2.9 morpholog etotal and progressive motility</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

• Infertile patients have higher sORP value when compared to fertile donors.
• Abnormal sperm parameters, especially two parameters, were found to be correlated with increased sORP values. One patient has 3 abnormalities and no patient had four abnormalities.
• Morphological abnormality was found almost all infertile patients. When combined with other abnormal parameters, only infertile group has both abnormalities. Fertile group has either morphology or other abnormality.
• Independent of other variables, as the % of neck abnormalities increased, sORP/Concentration was also increased. As the total number of sperm in a semen sample increased, sORP /Concentration tended to decrease but this decrease was very small.

Infertile men have a significant imbalance in the semen redox system, such that by measuring sORP, infertile patients and fertile donars can be distinguished by their sORP values. Measurement of sORP can be used to diagnose male infertility patients with abnormal semen parameters and it may help to optimize antioxidant treatment as well as evaluation of assisted reproduction.
SEMINAL OXIDATION-REDUCTION POTENTIAL CAN DIFFERENTIATE FERTILE FROM INFERTILE MEN

Introduction
Male factor can be the sole or contributing factor in roughly half of infertility cases. No identifiable cause can be found in over 25% of infertile males. Semen analysis is routinely used to evaluate the male partner in infertile couples and to assess the reproductive toxicity of environmental or therapeutic agents. Despite it being a routine analysis, it is a subjective interpretation of semen quality and is a poor predictor of fertility evidenced by the presence of unexplained infertility in men with normal semen analysis. These limitations call for an unbiased, quantitative measure to accurately differentiate fertile from infertile men and validate the subjective values of semen quality.

Oxidation-reduction potential (ORP) is a simple objective measure, newly applied to assess the status of oxidative stress in semen and has been evaluated in comparison with the WHO parameters (5th Edition, 2010). ORP may hold potential value in fertility assessment, as oxidative stress has been linked with infertility.

Materials and Methods
The study included 365 males from infertile couples and 50 fertile controls which had achieved pregnancy in the last 24 months. Static ORP (sORP) was measured using the MiOXSYS System (Figure 1).

Semen analysis was done according to the 5th Edition WHO manual (2010) after at least 2 days of abstinence. Static ORP (sORP) was measured using the MiOXSYS System (Figure 1).

Table 2. Basic demographic data for infertile patients and fertile controls.

Conclusions
Infertile patients had:
- Higher sORP values
- Fewer normal sperm morphology
- Lower sperm motility
- Lower normal sperm morphology

sORP can distinguish between infertile patients and fertile controls
sORP cutoff of greater than 1.42 mv/106 sperm/mL can predict if a semen sample came from infertile patients with a positive predictive value of 95.7% and a specificity of 78%.

Discussion
Measuring sORP provides an objective measure that relates to semen quality.

sORP, at a cutoff value of 1.42 mv/106 sperm/mL, provides an objective and repeatable measure for identification of patients more likely to be infertile.

This new test can rapidly help clinicians identify infertile patients with confidence, so that treatment strategies can be initiated immediately. This in turn helps the clinic improve the chances of pregnancy.
EVALUATION OF INTRA- AND INTER-OBSERVER RELIABILITY OF THE OXIDATION-REDUCTION POTENTIAL TEST FOR OXIDATIVE STRESS IN MALE FACTOR INFERTILITY

Ashok Agarwal, PhD., 1 Shubhadeep Roychoudhury, PhD., 1 Rakesh Sharma, PhD., 1 Sajal Gupta, MD., 1 Ahmad Majzoub, MD., 2 Kim Bjugstad, PhD., 3 and Edmund S Sabanegh., MD. 2

1 American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH. 2 Cleveland Clinic, Cleveland, OH. 3 Abyu Bioscience, Englewood, CO

ABSTRACT

Objective: Oxidative stress (OS) plays a role in male infertility. Because OS is a state in which oxidative activity exceeds antioxidative protection, a measure of both usual in the field using semen samples from normozoospermic men and women. Measuring ORP is a more approach to testing fertility as such the reproducibility of an ORP test is crucial for clinical application. The objective of this study was to evaluate the intra-observer and inter-observer reliability of the ORP test.

Materials and Methods: Semen ORP was measured using the MiOXSYS System. For intra-observer reliability, 2 samples were measured 3 times by observers using the same analyzer. The reliability was determined using the average coefficient of variation (%CV) across samples on an individual observer. For inter-observer reliability, 3 samples were measured 3 times by 3 different observers to determine the %CV. The reliability was determined by the difference between observer means, correlation, and %CV.

Results: Results of the intra- and inter-observer reliability experiments are summarized in Table 1. The overall %CV was 8.39% suggesting a strong level of intra-observer reliability. The %CV across observers was 3.61%, indicating a strong level of inter-observer reliability. Conclusion: Our results of the intra- and inter-observer reliability experiments confirm the reproducibility of the MiOXSYS System.

INTRODUCTION

Male infertility is a worldwide concern medical condition occurring in 15% of men globally. Oxidative stress (OS) resulting from an imbalance between oxidants and antioxidants is believed to play a central role in the pathogenesis of male infertility. A balance in the redox system is required for essential physiologic functions of the sperm such as capacitation, sperm-oocyte fusion, hyperactivation, acrosome reaction and sperm-oocyte fusion. Currently available assays for spermatozoa such as chromatin compaction in maturing spermatozoa, capacitation, monitoring the redox system and thus of OS. The ORP test is in the area of infertility. It is a novel test based on a galvanostatic measure of electrons — the MiOXSYS System – that measures the amount of oxidative or reductive stress (redox balance) in semen samples by measuring the ORP. After semen analysis and within one hour of collection, a 30-µL aliquot of liquefied semen was subjected to the ORP test using a novel galvanostat-based technology—the MiOXSYS System. The MiOXSYS System is comprised of a platinum-based anode and disposable sensor, measures the amount of oxidative or reductive stress (redox balance) in semen samples by measuring the ORP.

MATERIALS AND METHODS

After complete liquefaction, initial semen analysis was done to measure sperm concentration which is necessary in the calculation of ORP. After determining the sperm concentration, semen samples were diluted 1:4 with centrifuge supernatant and a 15 µL sample was added to the MiOXSYS® analyzer. The reliability was determined using the average coefficient of variation (%CV) across samples on an individual observer.

RESULTS

The intra- and inter-observer reliability of the ORP test

Table 1: The intra- and inter-observer reliability of the ORP test

<table>
<thead>
<tr>
<th>Observer 1</th>
<th>Observer 2</th>
<th>Observer 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>%CV</td>
<td>7.99%</td>
<td>5.72%</td>
<td>11.96%</td>
</tr>
<tr>
<td>FDR</td>
<td>0.0008</td>
<td>0.0183</td>
<td>0.0004</td>
</tr>
<tr>
<td>%CV intra-observer reliability</td>
<td>0.991</td>
<td>0.993</td>
<td>0.993</td>
</tr>
<tr>
<td>%CV inter-observer reliability</td>
<td>0.993</td>
<td>0.993</td>
<td>0.993</td>
</tr>
</tbody>
</table>

CONCLUSIONS

1. The results of the intra- and inter-observer reliability experiments confirm the reproducibility of the MiOXSYS System for use in clinical settings.
2. It is important to validate the new test instrument irrespective of its ease of use and simplicity. This is especially important when a system is used in a clinical setting to report patient results.
To learn more about MiOXSYS, visit us at www.mioxsys.com