ORIGINAL ARTICLE

Correspondence:
Ashok Agarwal, American Center for Reproductive Medicine, 10681 Carnegie Avenue, Cleveland Clinic, X-11, Cleveland, OH 44195, USA.
E-mail: agarwaa@ccf.org

A multicenter study to evaluate oxidative stress by oxidation–reduction potential, a reliable and reproducible method

[1]A. Agarwal 🆔, [2,3]M. Arafa, [1]R. Chandrakumar, [3]A. Majzoub, [3]S. AlSaid and [3]H. Elbardisi

[1]American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA, [2]Department of Andrology, Cairo University, Cairo, Egypt, and [3]Department of Urology, Hamad Medical Corporation, Doha, Qatar

SUMMARY

Seminal oxidative stress (OS) is well-known to affect male fertility status. The discrepancy in OS measurement has hindered its clinical use as a quality indicator for semen. Some tests measured single markers of oxidants or reductants, leading to lack of standardization of results. Oxidation–reduction potential (ORP) is a better representative for OS as it provides an overall measure of the activity of both oxidants and reductants. ORP assessment by MiOXSYS has been introduced as a measure of OS with high specificity in differentiating fertile from infertile semen samples. This is a retrospective study comparing data from semen analysis and ORP measurements between two andrology laboratories in the USA and Qatar over a period of 12 months. The same protocol was followed by both laboratories. The USA dataset contained 194 patients and 51 fertile donors, while the Qatar dataset contained 400 patients and 50 fertile donors. In both datasets and in the combined dataset, 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 ($p < 0.001$). Also, the percentage of patients with abnormal semen volume, sperm count, total and progressive motility were significantly different between both datasets ($p < 0.05$). ORP levels showed no significant difference between both datasets ($p < 0.08$). ROC analysis indicated that ORP cutoff value of $1.42 \text{ mV}/10^6/\text{mL}$ in the USA group, Qatar group, and combined dataset can accurately differentiate fertile from infertile semen groups. Although other semen parameters showed significant differences between the two centers, ORP remained consistent in both datasets individually or in combined data. This proves its reproducibility and reliability.

INTRODUCTION

Approximately 15% of couples fail to conceive a child after a year of regular, unprotected intercourse, and male factor is an attributable cause in 20–50% of these 48.5 million cases globally (Sharlip *et al.*, 2002; Agarwal *et al.*, 2015a).

Routine semen analysis is still used as the primary test to evaluate male fertility (Esteves, 2014). However, due to the high intra- and interassay variability, change in semen parameters over time, and lack of information about other sperm functional properties such as DNA fragmentation and OS, semen analysis is a poor predictor of men's fertility potential (Esteves, 2014; Björndahl *et al.*, 2016). Other potential markers of sperm quality such as DNA fragmentation and OS may be able to supplement routine semen analysis as indicators of infertility. OS has been shown to play an important role in the pathophysiology of male infertility (Pasqualotto *et al.*, 2000; Agarwal *et al.*, 2003, 2006, 2014a,b; Mahfouz *et al.*, 2010; Ghareeb & Sarhan, 2014; Dobrakowski *et al.*, 2017).

OS occurs when there is excessive production of reactive oxygen species (ROS) or deficiency of antioxidants, causing an imbalance between oxidants and reductants. Elevated levels of ROS in seminal plasma are present in 30–40% of infertile men and in up to 80% of idiopathic infertility cases (Agarwal *et al.*, 2014c). Low levels of antioxidants are also present in the seminal plasma of infertile men (Mahfouz *et al.*, 2009). At low physiological levels, there exists a balance in the redox state, and ROS are reactive oxygen metabolites that are necessary for optimal sperm functions such as motility, hyperactivation, capacitation,

acrosome reaction, and sperm–oocyte fusion (Agarwal *et al.*, 2014c). However, high levels of ROS produced by dysfunctional spermatozoa and leukocytes in the seminal plasma can have adverse effects on sperm function (Sharma & Agarwal, 1996; Agarwal *et al.*, 2014a,b,c). Due to sperm cell membranes being rich in polyunsaturated fatty acids, they are highly vulnerable to ROS, resulting in lipid peroxidation of the plasma membrane (Henkel, 2011). Other effects of high levels of ROS include lipid peroxidation of intracellular lipids and proteins, aggravation of apoptosis, and DNA damage, all of which result in decreased sperm quality and reduced fertility (Guthrie & Welch, 2012; Walczak-Jedrzejowska *et al.*, 2013; Aitken *et al.*, 2014, 2016).

Oxidative stress markers in male factor infertility are typically evaluated by single marker measurements that fail to capture both of the components of OS, oxidants, and reductants (Mahfouz *et al.*, 2009; Agarwal *et al.*, 2015a,b). Consequently, results from different tests can lead to inconsistent conclusions about the presence or absence of notable oxidative stress; lipid peroxidation assays results often do not correspond to results from antioxidant capacity tests or DNA fragmentation index (Dotan *et al.*, 2004). Individually, these tests do not provide the full picture of the true oxidative state of the sample and reflect only the redox status at a single point.

The MiOXSYS System is a novel technology based on a galvanostatic measure of electrons that has been recently used to assess changes in OS in trauma patients and as a function of extreme exercise (Azbill *et al.*, 1997; Powers & Jackson (2008). It has also been utilized to measure oxidation–reduction potential (ORP) in both semen and seminal plasma (Agarwal *et al.*, 2016b). It bypasses many of the challenges of traditional OS measurement methods as it requires 30 μL of sample (Agarwal *et al.*, 2016b). ORP measures the transfer of electrons, providing a comprehensive measure of both oxidants and antioxidants at one time. This replaces the need to measure each component separately. This method allows the oxidative state and thus the measure of OS, to be captured in real time. High levels of ORP indicate OS, and ORP levels have been shown to correlate negatively with semen parameters and can distinguish between infertile men and controls (Agarwal *et al.*, 2016b).

While previous studies conducted by our group have indicated the low technical variability and stable nature of ORP with samples over time, samples from the same individual and samples within a single laboratory (Agarwal *et al.*, 2016b, 2017; Agarwal & Wang, 2017), no current study has been conducted to investigate the consistency of the ORP measurement between laboratories or among different ethnic populations.

Therefore, the goals of this multicenter study was to investigate (i) the reproducibility and reliability of the ORP measurement as an indicator for sperm quality across different fertility centers and (ii) establish the ORP cutoff value to distinguish infertile men and healthy controls.

MATERIALS AND METHODS

Subjects

After approval by the Institutional Review Board for both institutes, semen samples were obtained from 51 normal fertile donors and 194 infertile patients at the Cleveland Clinic from August 2015 to August 2016. Samples were obtained from 400 infertile patients and 50 fertile donors (over the same time frame) from the male infertility unit of a large teaching hospital in Doha, Qatar. Infertility was defined as the inability to conceive after at least 12 months of regular unprotected intercourse. Both the patients and fertile donors from each location were classified based on normal or abnormal semen parameters according to the WHO 5th edition guidelines (World Health Organization, 2010). The infertile group was composed of patients presenting to male infertility units in both locations complaining of primary or secondary infertility. The exclusion criteria for both patients and controls were as follows: presence of azoospermia on semen analysis, evidence of obstructive pathology or ejaculatory dysfunction suggested by a low semen volume with/without an acidic pH, female factor infertility, and incomplete semen collection. The study population was divided according to the results of the semen analysis into normal and abnormal semen groups. The abnormal semen group had at least one of the following abnormal sperm parameters: semen volume <1.5 mL, sperm concentration $<15 \times 10^6$ sperm/mL, total sperm count $<39 \times 10^6$ sperm, total motility <40%, progressive motility <32%, or normal morphology <4%. Normal sperm parameters fell within the 2010 WHO normal reference ranges.

Semen analysis

The same protocol for semen analysis was followed by the two centers. Semen specimens were collected in sterile containers by masturbation after 48–72 h of sexual abstinence. After complete liquefaction at 37 °C for 20 min, sperm parameters were analyzed according to the WHO criteria (World Health Organization, 2010). Each sample was analyzed for macroscopic parameters such as color, pH, ejaculate volume, semen age (from collection to analysis), and viscosity. An aliquot of the sample (5 μL) was examined for sperm concentration, total sperm count, sperm motility, and round cell concentration. If the round cell concentration was $>1 \times 10^6$/mL, the sample was examined for the presence of white blood cells by the Endtz test using a MicroCell counting chamber (Vitrolife, San Diego, CA, USA) with phase optics set at ×20 magnification. Air-dried smears were prepared for morphological evaluation, and a total of 200 spermatozoa were scored, with 4% normal morphology used as a cutoff.

Measurement of oxidation–reduction potential

ORP was measured in millivolts (mV) using galvanostat-based technology (MiOXSYS® System; Aytu Bioscience, Englewood, CO, USA). A 30 μL sample suspension is loaded onto the sample port of the pre-inserted disposable sensor, and the measurement begins automatically. The test starts when the sample fills the reference electrode, thereby completing the electrochemical circuit. After a period under 4 min, the static ORP (sORP) values, in millivolts (mV), are displayed on the screen. Raw sORP values (mV) were normalized to sperm concentration—a value that reflects both semen volume and sperm number. Data for sORP are presented as mV/10^6 sperm/mL throughout. sORP provides a 'snapshot' of the current balance between total oxidants and reductants in a biological system. A higher sORP level indicates a greater imbalance with the activity of all available oxidants relative exceeding the activity of all available antioxidants in the

seminal ejaculate, reflecting a state of OS. The reproducibility of sORP was determined with multiple measurements of the same semen sample and across time through measurements of different semen samples obtained from the fertile group two weeks apart. For the reliability study of the ORP measurement, samples from the 50 proven fertile donors were measured in duplicate. After 2 weeks, measurements were repeated for the same individuals resulting in a total of four measurements per donor.

Statistical analysis

Data were analyzed separately from each laboratory and then was combined and analyzed. Data are presented as median (25th, 75th percentile). Comparisons of groups were performed using Fisher's exact test or chi-squared test for categorical variables such as frequency distribution of sperm parameters. A Wilcoxon rank-sum test was used for group comparisons with respect to quantitative variables such as volume, sperm concentration, total sperm count, percentage motility, sperm morphology and sORP (mV/10^6 sperm/mL). Results are reported as median (25th percentile, 75th percentile), and tests were performed at a significance level $p < 0.05$.

The analysis was redone after excluding patients with smoking history and evidence of leukocytospermia on semen analysis. Within this subset analysis, linear regression was performed to assess whether relationships between sORP (mV/10^6 sperm/mL) and each of infertility or sperm abnormality were preserved in the context of adjustments for age, BMI, and number of abstinence days. A receiver operating characteristic (ROC) curve was used to identify the sORP (mV/10^6 sperm/mL) criterion (cutoff, sensitivity and specificity, positive predictive value, negative predictive value, accuracy, and area under curve [AUC]) that best differentiated infertile patients from fertile donors for the Cleveland Clinic study population, the Qatar population, and a combination of the two datasets.

To establish the reliability of the ORP reading, intraclass correlation coefficients for consistency and absolute agreement in a two-way model for the same rater for all subjects was performed in 50 proven fertile donors. The means of both measurements were compared by means of the paired samples t-test.

RESULTS

Based on the combined dataset of Cleveland Clinic, USA, and Doha, Qatar study populations, sperm concentration, total motility, progressive motility, and normal morphological forms were significantly lower in infertile patients ($n = 594$) compared to fertile donors ($n = 101$) ($p < 0.001$), while ORP levels were significantly higher in infertile patients ($p < 0.001$) (Table 1).

The comparisons of sperm parameters and ORP levels among infertile patients between Cleveland Clinic ($n = 194$) and Doha ($n = 400$) male infertility units indicated there were no significant differences in volume, concentration, total motility, or ORP levels between patients in the two locations. However, significant differences were observed in progressive motility and morphology measurements (Table S1). In the case of the control populations between Cleveland Clinic ($n = 51$) and Doha ($n = 50$), the only significant difference in sperm parameters was seen in normal morphological forms. No significant differences were observed in volume, concentration, total motility, or ORP levels between controls (Table S2). In men with proven fertility, 52% still presented with at least one abnormal semen parameter

Table 1 Sperm parameters and ORP values (mv/10^6 sperm) in controls ($n = 101$) vs. patients ($n = 594$) for combined dataset of Cleveland Clinic and Doha study populations [values are presented as median (25th, 75th percentile)]

Variable	Control	Patients	p-value
Age (year)	32.3 ± 1.1	35.5 ± 0.4	0.52
BMI (kg/m^2)	28.5 ± 0.5	31.5 ± 1.6	0.13
Abstinence (days)	3.7 ± 0.2	4.4 ± 0.1	0.2
Infertility duration (year)	0 (0)	3.6 ± 1.8	na
Volume (mL)	3 (1.9, 3.4)	2 (3, 4)	0.012
Concentration (10^6 sperm/mL)	54 (33, 76)	22.5 (6, 47.08)	<0.001
Total motility (%)	59 (53, 65)	43 (25, 60)	<0.001
Progressive motility (%)	32 (20, 33)	15 (0, 31)	<0.001
Morphology (normal form %)	8 (5, 11)	3 (1, 9)	<0.001
ORP (mV/10^6 sperm)	1 (0.52, 1.64)	1.94 (0.9, 7.07)	<0.001

in semen analysis, while 18.1% of infertile patients presented with no abnormal semen parameters. Additionally, 47.4% of infertile patients in Cleveland Clinic presented with abnormal concentration compared to only 32.8% of Doha patients ($p = 0.001$), 53.4% of Cleveland Clinic patients presented with abnormal motility compared to 38.2% of Doha patients ($p = 0.001$), and 57.4% of Cleveland Clinic patients presented with abnormal progressive motility compared to 80.2% of Doha patients ($p < 0.001$).

By combining both the populations, groups were classified based on normal semen ($n = 114$) and abnormal semen ($n = 433$). ORP levels were elevated in abnormal semen ($p < 0.001$) (Table 2). Similar results were observed when infertile patients in both locations were differentiated by normal semen ($n = 90$) and abnormal semen ($n = 407$), summarized in Table 3.

Using MiOXSYS, at the cutoff value of 2.26 (mV/10^6 sperm), ORP was able to differentiate between infertile patients and fertile controls in the Cleveland Clinic study population with 49.0% sensitivity, 84.3% specificity, and 92.2% positive predictive value (Fig. 1A). However, at a lower cutoff value of 1.42 (mV/10^6 sperm), ORP was able to differentiate between infertile patients and fertile controls in the Doha study population with 60.8% sensitivity, 78.0% specificity, and 95.7% positive predictive value (Fig. 1B). Therefore, by analyzing the combined dataset, we were able to established an ORP cutoff value of 1.42 (mV/10^6 sperm) with 60.6% sensitivity, 74.3% specificity, and 93.3% positive predictive value (Fig. 1C).

Among infertile patients from Cleveland Clinic, the incidence of leukocytospermia (leukocytes $>1 \times 10^6$/mL) was 12.3% (36/293), and the incidence of leukocytospermia among Doha patients was 9.3% (37/400). All fertile donors were non-smokers, while 26.3% of patients had a history of smoking. After excluding

Table 2 Sperm parameters and ORP values (mv/10^6 sperm) in subjects ($n = 547$) with at least one abnormal sperm parameter for combined dataset of Cleveland Clinic and Doha study populations [values are presented as median (25th, 75th percentile)]

Variable	Normal sperm ($n = 114$)	Abnormal sperm ($n = 433$)	p-value
Volume (mL)	3 (2, 3.8)	3 (2, 4)	0.91
Concentration (10^6 sperm/mL)	55.3 (40, 80)	20 (5, 40.8)	<0.001
Total motility (%)	64.5 (62, 72)	40 (20, 53)	<0.001
Progressive motility (%)	35 (32, 40)	10 (0, 20)	<0.001
Morphology (normal form %)	13 (9, 18.5)	3 (1, 6)	<0.001
ORP (mV/10^6 sperm)	0.85 (0.51, 1.14)	2.27 (1.09, 8.77)	<0.001

Table 3 Sperm parameters and ORP values (mv/10⁶ sperm) in infertile patients (*n* = 497) with at least one abnormal sperm parameter for combined dataset of Cleveland Clinic and Doha study populations [values are presented as median (25th, 75th percentile)]

Variable	Normal sperm (*n* = 90)	Abnormal sperm (*n* = 407)	*p*-value
Volume (mL)	3 (2, 3.75)	3 (2, 4)	0.21
Concentration (10⁶ sperm/mL)	54.8 (40, 80)	19 (4.96, 39)	<0.001
Total motility (%)	65 (62, 72)	40 (20, 52)	<0.001
Progressive motility (%)	35 (32, 43)	9 (0, 20)	<0.001
Morphology (normal form	15 (9, 20)	3 (1, 6)	<0.001
ORP (mV/10⁶ sperm)	0.83 (0.51, 1.11)	2.49 (1.15, 10.16)	<0.001

subjects with a history of smoking and with leukocytospermia, the combined data revealed the same ORP cutoff value of 1.42 (mV/10⁶ sperm) with 58.8% sensitivity, 78.0% specificity, and 93.7% positive predictive value (Figure S1). ROC analysis was also performed in this subset to distinguish subjects with at least one abnormal semen parameter from those with zero abnormal semen parameters at an ORP cutoff value of 1.4 (mV/10⁶ sperm) with 68.6% sensitivity, 79.6% specificity, and 93.3% positive predictive value (Figure S2). This subset of subjects (non-smokers without leukocytospermia) presented with differences between semen parameters in fertile controls and infertile patients similar to those of the combined dataset (Table S3).

Linear regression analyses were performed to adjust for age, BMI, and number of days of abstinence with ORP as the dependent variable and infertility among all subjects (Table S4) or sperm abnormality among patients as an independent variable (Table S5). While the ROC curves do not adjust for age, BMI, and number of days of abstinence, these regression analyses showed highly significant relationships between ORP and either infertility or sperm abnormality, even with adjustments of covariates. Therefore, these results demonstrate that the associations between ORP and infertility exist independently of smoking status, leukocytospermia, age, BMI, and number of abstinence days.

In the fertile donor group, there were no statistically significant differences in multiple measurements of ORP measurements from the same semen samples (Table S2). Moreover, insignificant differences ORP levels were also detected across time in samples obtained 2 weeks apart (1.23 ± 1.03 mV/10⁶ sperm in measurement 1 and 1.13 ± 0.85 mV/106 sperm in measurement 2, *p* = 0.68).

Results of the intraclass correlation of the repeated ORP results to determine the reliability of the ORP measurement showed both, good consistency and absolute agreement, and are depicted in Table S6. Data distribution for each measurement is shown in Figure S3. The mean values of both measurements, 56.5 ± 2.8 mV/10⁶ sperm and 54.0 ± 2.5 mV/10⁶ sperm, respectively, showed no differences (*p* = 0.2286).
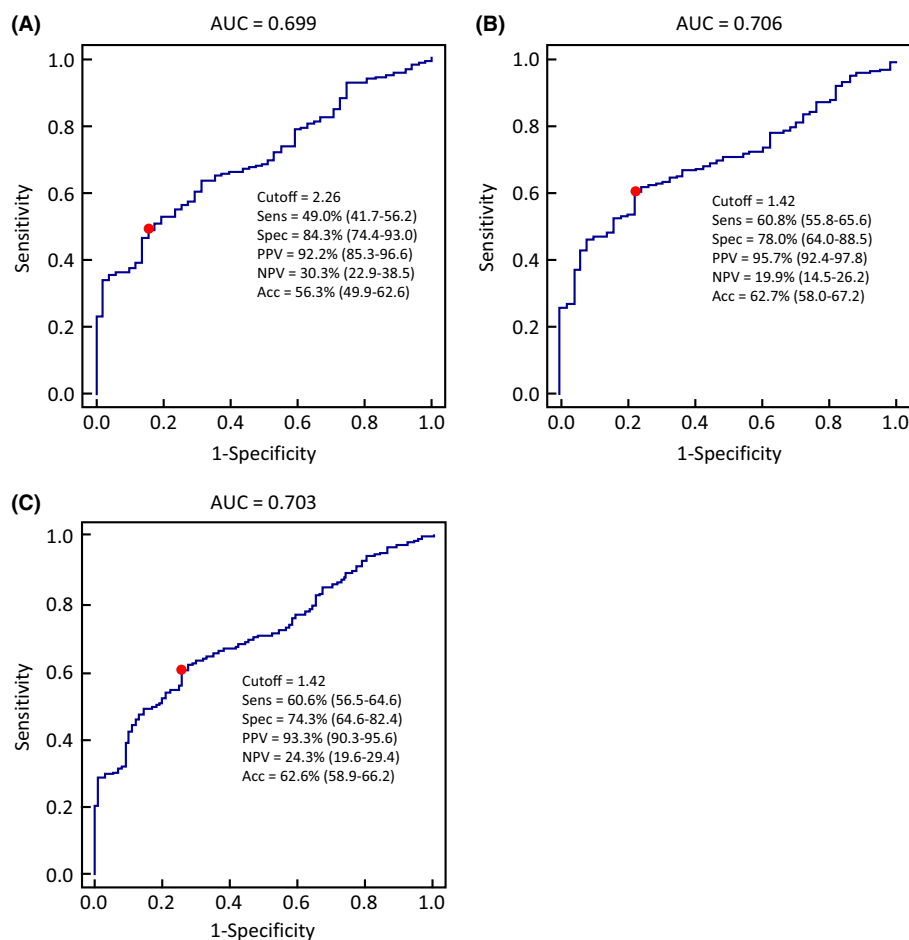

(A) AUC = 0.699

Cutoff = 2.26
Sens = 49.0% (41.7-56.2)
Spec = 84.3% (74.4-93.0)
PPV = 92.2% (85.3-96.6)
NPV = 30.3% (22.9-38.5)
Acc = 56.3% (49.9-62.6)

(B) AUC = 0.706

Cutoff = 1.42
Sens = 60.8% (55.8-65.6)
Spec = 78.0% (64.0-88.5)
PPV = 95.7% (92.4-97.8)
NPV = 19.9% (14.5-26.2)
Acc = 62.7% (58.0-67.2)

(C) AUC = 0.703

Cutoff = 1.42
Sens = 60.6% (56.5-64.6)
Spec = 74.3% (64.6-82.4)
PPV = 93.3% (90.3-95.6)
NPV = 24.3% (19.6-29.4)
Acc = 62.6% (58.9-66.2)

Figure 1 AUC/ROC of ORP (mv/10⁶ sperm) in distinguishing infertile patients from healthy controls in (A) Cleveland Clinic, USA dataset, (B) Doha, Qatar dataset, (C) combined dataset. [Colour figure can be viewed at wileyonline library.com].

DISCUSSION

Although routine semen analysis is still used as the basis for male infertility evaluation, high technical and biological variability raise questions about the reliability of this test Esteves, 2014; Jarow *et al.*, 2013; Filimberti *et al.*, 2013). It does not provide information about fertility potential Wang & Swerdloff, 2014); however, if infertile men present with normal sperm parameters during semen analysis, clinicians may be quick to diagnose these men with unexplained infertility. Additionally, concerns have been raised that subfertile men may be misclassified as fertile as a result of the lowered reference values of the WHO 5th edition 2010 guidelines compared to the 4th edition guidelines (Murray *et al.*, 2012). The consistency of semen analysis results is further compromised by an overwhelming majority of laboratories failing to adhere to the WHO guidelines (Mallidis *et al.*, 2012). Considering the limited and oftentimes inconsistent results of semen analysis, it is important to add other supplementary tests to semen analysis to provide a more comprehensive and accurate fertility assessment.

Oxidative stress is a key mediator in the pathogenesis of male infertility. Around 30–80% of infertile men present with elevated ROS levels, and they tend to also present with lower total antioxidant capacity levels (Mahfouz *et al.*, 2009; Agarwal *et al.*, 2014a, b,c). However, clinicians have been reluctant to include oxidative stress measurements as part of a routine diagnostic workup due to the lack of a standardized protocol used and obscurity about ROS reference values (Sikka & Hellstrom, 2016). The current methods for measurement of OS are characterized by a combination of independent assays (Kashou *et al.*, 2013). ROS is measured in semen by chemiluminescence assays, and total antioxidant capacity (TAC) is measured in seminal plasma by a colorimetric assay. The results of ROS and TAC tests could be combined to generate a composite ROS-TAC score (Sharma *et al.*, 1999; Mahfouz *et al.*, 2009; Agarwal *et al.*, 2015b). Other methods for measuring OS include the malonaldialdehyde assay, which assesses the levels of a lipid peroxidation marker, as well as cytochrome c reduction, nitroblue tetrazolium reduction, and electron spin resonance spectroscopy (Grotto *et al.*, 2007; Agarwal & Sekhon, 2011). While these traditional methods are useful, they are time-sensitive and time-consuming (45 min), making them unsuitable for routine diagnostic purposes. They also require expensive, highly complex instruments and are limited to fresh specimens and large sample volumes (Agarwal *et al.*, 2016a).

Contrary to single marker assessments, ORP provides a comprehensive measure of oxidative stress. It involves the analysis of all known and unknown oxidants and antioxidants in the given sample and does not depend on any one measurement to be able to extrapolate the true reflection of the level of oxidative stress. The novel MiOXSYS system yields ORP results that eliminate many of the limitations posed by semen analysis and conventional oxidative stress assays. The test takes <4 min, is inexpensive, and requires only 30 μL of sample. The MiOXSYS System eliminates the high intra- and interobserver variability seen in semen analysis and allows for ORP in both semen and seminal plasma to be accurately measured at least 120 min after collection (Agarwal *et al.*, 2016b, 2017). ORP has also been shown to be strongly negatively correlated with concentration, count, and motility in infertile patients (Agarwal *et al.*, 2016b).

Our study correlates with these previous findings as significantly decreased values in concentration and motility corresponded to significantly increased values of ORP levels in infertile patients compared to healthy controls and increased ORP levels in men with abnormal semen parameters compared to men with normal semen parameters.

It is important to note that there has been a global downward trend in semen quality over the last several decades, although the specific trends within ethnic or regional populations are not well studied (Merzenich *et al.*, 2010). Studies have shown that there are regional and continental variations in sperm parameters of semen analysis (Jørgensen *et al.*, 2001; Nallella *et al.*, 2006). In our study, both laboratories are strictly adherent to WHO 2010 criteria for semen analysis. However, we found significant differences between the Doha and Cleveland Clinic datasets in the infertile groups in progressive motility and normal morphology and in the fertile groups in normal morphology. In quality control programs, morphology is the parameter with the highest variability between laboratories, which may explain the significant difference in normal morphological forms observed in the two study populations (Filimberti *et al.*, 2013). The percentage of patients with sperm count, total motility, and progressive motility were also significantly different between both the Cleveland Clinic and Doha study populations. Although significant differences between the parameters were present, ORP levels for both infertile patients and healthy controls showed no significant differences between the two study populations. This demonstrates when other sperm parameters may not be consistent between laboratories, ORP remains stable and is thus a reproducible and reliable measure of sperm quality.

We demonstrated that ORP was able to accurately differentiate between infertile men and healthy controls in the combined dataset at a cutoff value of 1.42 mV/10 sperm with 60.6% sensitivity and 74.3% specificity. This cutoff value was identical to the Doha optimal cutoff value and was lower than the Cleveland Clinic optimal cutoff value of 2.26 mV/10^6 (49.0% sensitivity and 84.3% specificity). The Doha population had greater influence on the combined result due to its larger sample size, and when a cutoff value of 1.42 mV/10^6 sperm was applied to the Cleveland Clinic dataset, the sensitivity and specificity reflected similar values. Previous studies conducted by our group have established ORP levels of 1.48 mV/10 sperm to distinguish abnormal motility from normal motility (Agarwal *et al.*, 2016b), 1.36 mV/10^6 sperm to distinguish infertile patients from healthy controls (Agarwal *et al.*, 2017), and 1.57 mV/10^6 sperm to detect at least one abnormal sperm parameter (Agarwal *et al.*, 2017). The consistency of the cutoff value with these previously established optimal cutoff values indicates that ORP is a highly reliable and reproducible measure of sperm quality and provides useful information about the oxidative state of samples from infertile men. It also provides a preferable alternative to ROS assays with ill-defined reference values.

The limitations of our study include the following: (i) The study had a retrospective design, although the same protocol was followed by both centers, and (ii) Varicocoele was not studied as an independent variable in this study; therefore, patients and fertile donors with varicocoele were not excluded. (iii) While semen parameters are an important part in the assessment of

the infertile male, the gold standard is the ability to determine the reproductive outcome. Pregnancy outcomes were not prospectively measured in the infertile group.

Previous studies by our group have established that ORP by MiOXSYS minimizes intra- observer and interobserver variability, correlates negatively with semen parameters, allows for reliable measurements in both semen and seminal plasma at least 120 min after collection, predicts poor sperm quality over time, and predicts oligozoospermia with high accuracy (Agarwal *et al.*, 2016b, 2017, 2017). While the simplicity of the test and its stable results have been shown within samples and among different samples in our laboratory, no studies have investigated the consistency of the test between different laboratories among different ethnic populations where semen parameters are often inconsistent.

In conclusion, this study reveals that the measurement of ORP in semen using the MiOXSYS System is a viable alternative method for measuring OS and distinguishing healthy controls from infertility patients. 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 sperm parameters, and it therefore can be used as a supplementary test to semen analysis to confirm poor semen quality or as a possible diagnostic tool for assessing infertility. A cutoff value of 1.42 mV/10^6 sperm was able to distinguish between male factor infertility patients and healthy donors with 60.4% sensitivity, 74.3% specificity, and 93.3% positive predictive value. The use of ORP as the primary marker for OS will allow for comparisons of OS-related studies, possible interventions and treatments of OS, and practical clinical application of OS as part of the standard infertility workup. Further studies are necessary to establish the relationship between ORP levels and fertilizing capacity as well as pregnancy outcomes. 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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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 AUC/ROC of ORP (mv/10^6 sperm) in distinguishing fertile donors from infertile males after excluding subjects with either a history of smoking or leukocytospermia.

Figure S2 AUC/ROC of ORP (mv/10^6 sperm) in distinguishing subjects with at least one abnormal semen parameter from those with no abnormal semen parameter.

Figure S3 Data distribution results of 4 individual ORP measurements (mV/10^6 sperm) taken 2 weeks apart and read in duplicate.

Table S1 Sperm parameters and ORP values (mv/10^6 sperm) in infertile patients for Cleveland Clinic ($n = 194$) vs. Doha ($n = 400$) study populations [values are presented as median (25th, 75th percentile)].

Table S2 Sperm parameters and ORP values (mv/10^6 sperm) in controls for Cleveland Clinic ($n = 51$) vs. Doha ($n = 50$) study populations [values are presented as median (25th, 75th percentile)].

Table S3 Sperm parameters and sORP values (mv/10^6 sperm) in controls ($n = 50$) vs. patients ($n = 327$) for combined dataset of Cleveland Clinic and Doha study populations, excluding smokers and subjects with leukocytospermia.

Table S4 Linear regression analysis for ORP as a function of infertility, age, BMI, and number of days abstinent for subset of non-smokers without leukocytospermia in combined dataset.

Table S5 Linear regression analysis for ORP as a function of sperm abnormality, age, BMI, and number of days abstinent for subset of non-smokers without leukocytospermia in combined dataset.

Table S6 Intraclass correlation coefficients for ORP the measurement of the 50 proven fertile donors to determine the reliability of the measurement for consistency and absolute agreement.