Semen quality and infertility status can be identified through measures of oxidation–reduction potential

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Summary
Standard analyses for evaluating semen quality require technical expertise and are interpretive in nature. Oxidative stress (OS) alters many of the semen parameters; thus, a measure of OS could be an indicator of semen quality. Static oxidation-reduction potential (sORP) is a universal measure of OS traditionally used in environmental applications but is increasingly used in biomedical studies. sORP was measured to determine how well it associates with semen quality and if it differentiates semen from infertile patients and fertile donors. All study participants (Infertile, n = 365 and Fertile, n = 50) underwent standard semen analyses, and sORP was measured in unprocessed semen. In infertile patients, sORP increased with decreased total sperm number, motility and morphology. sORP values were higher in samples with abnormal quality (low number, motility and/or normal morphology) compared with those of normal quality. Infertile patients had higher sORP values compared to fertile donors. A sORP cut-off value of 1.38 mV/10^6 sperm/ml can differentiate normal from abnormal semen samples, while a cut-off value of 1.41 mV/10^6 sperm/ml, can differentiate between infertile and fertile semen samples. In conclusion, sORP provides a quick and unbiased indicator of semen quality that can be a beneficial addition to semen analysis to determine semen quality and fertility status.

KEYWORDS
infertility, oxidation-reduction potential, semen analysis, semen quality, spermatozoa

1 | INTRODUCTION
Semen analysis is the cornerstone for assessment of male fertility. According to the World Health Organization’s (WHO) criteria for semen quality (WHO, 2010), there are several parameters of semen quality; however, sperm number and motility (total and progressive) carry the most weight as the status of these parameters.

It is generally accepted that the time and expertise required to do a full semen analysis, especially in a smaller laboratory, is often not cost effective (Wyrobek et al., 1997). It is tempting then, to rely on the results of only one or two semen parameters; however, Wang and Swerdloff stated that a single parameter cannot be used as a valid biomarker of fertility due to presence of multiple factors contributing to infertility (Wang & Swerdloff, 2014). A study of 378 couples trying to conceive naturally confirmed that no single semen parameter independently predicted fertility. However, data derived from the combination of parameters was able to give an insight into the likelihood of success (Buck Louis et al., 2014). It would be beneficial, then, to have a single measure of quality that reflects the combined influence of multiple parameters. Measuring imbalances in the reduction–oxidation (redox) system might qualify as this holistic quality measure.

The redox system balances the activity of oxidants and antioxidants (reductants) based on physiological need (Costantini & Verhulst, 2009; McCord, 2000). In males, oxidants like reactive oxygen species (ROS) and reactive nitrogen species have physiological roles in testicular immunity, spermatogenesis, motility, capacitation and other
functions (Doshi, Khullar, Sharma, & Agarwal, 2012; Ko, Sabanezh, & Agarwal, 2014). When the production of oxidants exceeds what is physiologically needed and the available antioxidants cannot mitigate their effects, a state of oxidative stress occurs. Oxidative stress has been associated with poor morphology, low motility and decreased number of spermatozoa (Benedetti et al., 2012; Hosseinzadeh Colagar, Karimi, & Jorsaraei, 2013; Khosrowbeygi & Zarghami, 2007; Walczak-Jedrzejowska, Wolski, & Slowikowska-Hilczer, 2013). Thus, it could be used as a biomarker for overall semen quality.

Previous attempts to use oxidative stress as an indicator of semen quality measured single features of the redox system measuring only ROS, specific antioxidants or post hoc oxidative damage (Jedrzejowska, Wolski, & Slowikowska-Hilczer, 2013). Thus, it could be ambiguous overall conclusions. A better measure of the redox system is the oxidation-reduction potential (ORP), which provides a measure of the activity of both the oxidants and antioxidants (Costantini & Verhulst, 2009; Shapiro, 1972). Measuring ORP as an indicator of the redox balance has been available since the 1930’s when it was first used as an indicator of water quality and later accepted by the WHO for drinking water standards (Schmelke, 1933; WHO, 2006, 2011). When applied to biomedical studies, changes in ORP values reflect the function of the redox system (Shapiro, 1972).

Traditional methods for measuring ORP use re-usable electrodes contained within glass probes submerged in large sample volumes. The major limitations of this technology have been electrode contamination and sample volume (Copeland & Lytle, 2014). Because of this, the application of ORP to biomedical assessment has been slow, with few studies (Rael et al., 2007, 2009; Zhi et al., 2013). Recently, the development of a novel technology has removed the limitations by creating a single-use electrode sensor that requires only 30 μl of sample. This has allowed expansion into biomedical fields in which oxidative stress is suspected (Agarwal, Sharma, Roychoudhury, Du Plessis, & Sabanezh, 2016; Bjugstad, Rael, et al., 2016; Bjugstad, Fanale, 2016; Rael, Bar-Or, Kelly, Carrick, & Bar-Or, 2015).

In this study, we have applied this technology to better explore the relationship of ORP with semen quality. To that end, we aimed to determine if ORP measures are related to individual parameters of semen quality in infertile men, specifically total sperm number, motility and morphology and if ORP can distinguish between semen from infertile men and men of proven fertility.

## 2 | MATERIALS AND METHODS

The prospective study was approved by the Institutional Review Board, and appropriate consents were signed by the patients before enrolment in the study.

### 2.1 | Infertile patient population

Men attending a specialised male fertility unit in a tertiary medical centre were recruited. The exclusion criteria were as follows: azospermia, antioxidant therapy, pyospermia, palpable varicocele, occupational chemical/radiation exposure, sperm concentration <1 million sperm/ml or presence of female factor infertility. All female partners had proven normal fertility status through a detailed medical report from their gynaecologists after performing full clinical examination and investigations for fertility.

### 2.2 | Fertile donor population

Men with proven fertility, that is having established a pregnancy in the last 24 months, were recruited through an advertisement placed by the same tertiary medical centre. Controls provided a proof of pregnancy in the past 24 months including child’s birth certificate and a medical report from the spouse’s gynaecologist stating that the pregnancy was spontaneous. The same exclusion criteria were applied.

### 2.3 | Data collection

General demographics were collected on all participants including age and body mass index (BMI).

Each participant provided a semen sample after ≥2 days of sexual abstinence. Standard semen analysis was performed using the 5th Edition WHO manual (WHO, 2010). Sperm concentration and motility were performed manually using a hemocytometer. Morphology was assessed by a single experienced technician based on spermatozoa stained using the Diff-Quik protocol. Kruger’s strict criteria were used for morphology assessment.

ORP was measured in duplicate in unprocessed post-liquefied semen to assess test reproducibility using the MiOXSYS System (Agarwal et al., 2016). The MiOXSYS System is comprised of an electrochemical analyser (Aytu BioScience, cat. # 100229) and single-use disposable semen sensors (cat. # 100283). Because the analyser applies a low voltage steady current measured in millivolts (mV), ORP is represented as the static ORP (sORP, mV). Oxidative stress reflects the relationship between spermatozoa (producers of free radicals) and seminal plasma (an antioxidant reservoir); thus, raw sORP values (mV) were normalised 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.

### 2.4 | Statistics

Comparisons of groups were performed using Fisher’s exact test or chi-square test for categorical variables such as frequency distribution of abnormal sperm count, abnormal motility and abnormal morphology. Wilcoxon’s rank sum test was used for group comparisons with respect to quantitative variables such as age, abstinence, volume, sperm concentration, total sperm count, per cent motility, sperm morphology and sORP between fertile and infertile group. Relationships between sORP and semen parameters or sORP and fertility were determined using Spearman’s correlation. For group comparisons, samples were grouped into “normal” or “abnormal” semen parameters and fertile and infertile men. Receiver operator characteristic (ROC) analyses with Youden's
Indices were used to determine cut-off values for sORP. The area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV) with their respective 95% confidence intervals (95% CI) were reported. Summaries of quantitative variables are in the form mean ± SE. Tests were performed at a significance level $p = .05$. Analyses were performed using R version 2.15.1.

### 3 | RESULTS

Final data set used for statistical analyses was 365 infertile patients and 50 fertile controls. Infertility was primary in 254 (69.6%) patients and secondary in 111 (30.4%) with a mean duration 3.2 ± 1.2 years. Table 1 presents the demographic data and overall semen analysis results. sORP did not significantly correlate with age ($r = -0.03$), BMI ($r = -0.04$) or abstinence ($r = -0.0008$).

<table>
<thead>
<tr>
<th>sORP/concentration (mV/10⁶/ml)</th>
<th>5.00 ± 0.56 (min–max = 0.06–131.67)</th>
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<tr>
<td>In fertile donors</td>
<td>1.26 ± 0.15 (min–max = 0.6–5.75)</td>
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$\text{Values are mean ± SE.}$

$p$ value $< .05$ was considered significant by Wilcoxon rank sum test for pairwise comparison between fertile donors and infertile men.

Using the collective status of sperm number, motility and morphology, data from infertile patients were grouped into normal semen quality ($n = 41$; met WHO parameters) and abnormal semen quality ($n = 324$; failed one or more). Those with abnormal quality semen had significantly higher sORP values than those with normal quality (5.49 mV/10⁶/ml versus 1.03 mV/10⁶/ml, respectively, $p$ value $< .02$).

ROC analysis indicated that sORP can accurately identify abnormal semen quality, as indicated by a significant coverage of area under the curve (AUC = 0.78, $p < .0001$). The associated sORP criteria for identifying and predicting abnormal quality were $>1.38$ mV/10⁶/ml (Figure 2) with sensitivity of 63.3%, specificity 87.8%, PPV 97.6% and NPV 23.2% and accuracy of 66%. The odds ratio that a semen sample with a sORP value greater than 1.38 mV/10⁶/ml was abnormal in quality was 10.05 ($p < .001$).

When comparing fertile donors to infertile patients’ group, we found that on average the fertile donors were significantly younger, had a shorter abstinence, but had similar BMI values (Table 2). As correlations between sORP and these variables, age and abstinence, were not significant (age: $r = -0.03$, abstinence: $r = -0.0008$, $p > .05$ both), no further analyses were carried out. As would be expected, fertile controls also had significantly higher concentration, total sperm numbers, higher sperm motility, and better sperm morphology than the infertile patients (Table 2). Only volume was similar between the groups.

sORP values measured in infertile patients ($n = 365$), regardless of semen quality, were significantly higher compared to those measured in donors of proven fertility ($n = 50$; ORP 5 versus 1.26 mV/10⁶/ml, $p$ value $< .02$). In a ROC analysis, sORP was able to significantly discriminate between semen samples (AUC = 0.68, $p < .001$). The optimal cut-off value was $>1.41$ mV/10⁶/ml. Those samples from fertile donors reliably fell below this sORP cut-off (specificity 78.0). The odds ratios

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<tr>
<td></td>
<td>In fertile patients</td>
</tr>
<tr>
<td>N</td>
<td>365</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.5 ± 0.4</td>
</tr>
<tr>
<td>BMI</td>
<td>31.5 ± 1.6</td>
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<tr>
<td>Abstinence (days)</td>
<td>4.4 ± 0.1</td>
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<tr>
<td>Volume (ml)</td>
<td>3.1 ± 0.08</td>
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<tr>
<td>Concentration ($\times 10^6$/ml)</td>
<td>36.2 ± 1.6</td>
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<tr>
<td>Total sperm number ($\times 10^6$)</td>
<td>110.8 ± 5.5</td>
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<tr>
<td>Total motility (%)</td>
<td>45.5 ± 1.2</td>
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<tr>
<td>Progressive motility (%)</td>
<td>27.0 ± 1.4</td>
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<tr>
<td>Morphology (%)</td>
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that semen sORP values greater than the cut-off of 1.41 mV/10⁶/ml were from infertile patients was 4.75 (p < .001).

Comparing sORP to individual WHO parameters for predicting semen from infertile patients indicated that progressive motility had a slight advantage over sORP with a higher accuracy but a lower PPV (Table 2). All other individual parameters were significant but, at the WHO cut-off values, were poor predictors (Table 2).

4 | DISCUSSION

Semen quality is a surrogate indicator of fertility; however, quality is usually based on the interpretation of an assembly of measured parameters. The current study is the largest one discussing sORP and semen analysis up till now with 365 infertile patients and 50 fertile donors. The data presented suggest that sORP could be a reliable measure of semen quality. sORP increased as individual semen parameters decreased. When the parameters were assessed collectively for semen quality, sORP could distinguish between those of abnormal and normal semen quality at the cut-off value of 1.38 mV/10⁶/ml. Further, sORP could discriminate between semen from infertile patients and fertile donors quality at the cut-off value of 1.41 mV/10⁶/ml.

Our results also confirm that, in general, individual semen parameters are poor predictors of infertility (Buck Louis et al., 2014; Wang & Swerdloff, 2014). sORP was better than total sperm number, total motility and morphology in identifying semen from infertile patients. Progressive motility was the only individual parameter that, in the current data set, could also identify infertile patients; however, progressive motility may not always be so reliable in other data sets. Furthermore, assessment of progressive motility is subjective, and operator dependent, therefore, its reliability as fertility marker is questionable. Moreover, in establishing the WHO criteria, Cooper et al. found that both total and progressive motility were lower in proven fertile donors (Cooper et al., 2010), suggesting a more complex relationship between motility and infertility.

Because sORP is a measure of oxidative stress and oxidative stress affects sperm numbers, motility and morphology (Agarwal et al., 2014; Benedetti et al., 2012; Haghighian et al., 2015), it would be reasonable to use sORP as a marker to support the interpretation of standard semen analyses. Previous studies attempted to relate oxidative stress and fertility using measures from only one side of

FIGURE 1  Higher sORP values were measured in abnormal parameters. (a) Semen with abnormally low numbers of spermatozoa had higher sORP values. (b, c) Semen with abnormally few motile spermatozoa was also related to higher sORP values. (d) Semen with fewer morphologically normal spermatozoa had higher sORP values. *, significant at p < .05

FIGURE 2  sORP values can distinguish and identify those semen samples that have abnormal quality. The ROC curve represents the area under the curve (AUC) that was occupied by different sORP cut-off values (AUC = 0.78*). The optimal sORP cut-off was 1.38 mV/10⁶/ml (circle marker). Black dashed line in A is at the 1.38 cut-off level. *, significant at p < .05

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into changes in semen and sperm quality than systemic elevations. The sORP reflect site-specific changes in oxidative stress (Benedetti et al., privilege status of the testes supports the notion that changes in semen fertility within the past 2 years. Another major difference between the proven fertility, while in the present study the controls have proven our study. However, Agarwal et al. included healthy controls with no value differentiating infertile from control group was 1.36 mV/10^6/ ml to differentiate normal and abnormal semen parameters which are slightly higher than the cut-off value in the present study. However, these authors used motility only as an indicator of semen quality while in our study abnormal semen was defined as failure to meet WHO 2010 criteria in any of the count, motility, progressive motility or normal morphology. The second study included 106 patients and 51 healthy controls (Agarwal et al., 2016). In this larger study, the cut-off value differentiating infertile from control group was 1.48 mV/10^6/ ml which is again slightly different from the cut-off value presented in our study. However, Agarwal et al. included healthy controls with no proven fertility, while in the present study the controls have proven fertility within the past 2 years. Another major difference between the present study and the previous report by Agarwal and coworkers is the number of patients (365 versus 106 and 33 patients). However, all the studies agreed on the correlation of sORP with semen parameters with nearly the same cut-off values.

The sORP values measured in semen did not change because of age or BMI; thus, the sORP values reflect the state of oxidative stress in the male reproductive system and not an indirect systemic measure of the body, which would distort the interpretation regarding oxidative stress in infertility. With our study, we could confirm the recent study by Agarwal et al. (2016) that sORP is not affected by age. The immunoprivilege status of the testes supports the notion that changes in semen sORP reflect site-specific changes in oxidative stress (Benedetti et al., 2012; Chen, Deng, & Han, 2016). Elevations in oxidative stress at the site of spermatogenesis and maturation are more likely to translate into changes in semen and sperm quality than systemic elevations. The ability to measure those small site-specific elevations through semen sORP helps to focus the relationship between oxidative stress, semen quality, and by extension-infertility.

The major limitation of the study is that the data comes from a single fertility centre. As the test is newly introduced for the assessment of semen, its reproducibility could be confirmed by comparing results from other centres. Another limitation is the lack of comparison of ORP to other methods of assessment of oxidative stress; however, as other methods are not validated and only measures one part of the oxidative equation, no sound comparison is expected. Although the study results revealed that sORP was slightly inferior to progressive motility in differentiating fertile from infertile semen samples, the objectivity and reproducibility of sORP favours its use in conjunction with conventional semen parameters for fertility prediction.

### 5 | CONCLUSIONS

sORP increased when individual parameters of semen quality were abnormal. sORP was also able to distinguish between semen with overall abnormal quality from those with normal quality, indicated by decreased sperm number, normal morphology and/or sperm motility. The difference between normal quality and abnormal quality was large enough to identify a reliable sORP cut-off value where semen with a sORP value greater than 1.38 mV/10^6/ml were 10 times more likely to have abnormal semen quality in infertile men. Similarly, the difference in sORP values between infertile patients and fertile donors was also large, such that a semen sample with a sORP value greater than 1.41 mV/10^6 sperm/ml was four times more likely to have come from an infertile patient than a fertile donor. Using sORP measures in conjunction with standard semen analysis could substantiate the result of the analysis and augment any conclusions drawn on a man’s fertility status.

### ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Dr. Bjugstad was a former employee of Aytu BioScience, Inc. The remaining co-authors declare no competing interests.

REFERENCES


SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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