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ORIGINAL ARTICLE

Male Fertility

Correlation of oxidation reduction potential and total motile sperm count: its utility in the evaluation of male fertility potential

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Oxidative stress (OS) is detrimental to sperm functions, and the oxidation reduction potential (ORP) is a good measure of OS as it considers the balance between oxidants and reductants. Total motile sperm count (TMSC) is viewed as the single most important semen analysis parameter that can predict male infertility severity, and its correlation with ORP has never been undertaken. The objectives of this study were to assess the correlation between ORP and TMSC, to identify the ORP cutoff value based on the TMSC result, and to compare this cutoff value with previously reported ORP cutoff values in literature. One thousand one hundred and sixty-eight infertile patients and 100 fertile controls were enrolled. Demographic and semen data of the participants were retrieved and analyzed. Wilcoxon's rank-sum test compared variables between infertile men and fertile controls; Spearman's correlation assessed the static ORP (sORP)-TMSC relationship for the whole sample and among each group individually. Using a 20×10^6 TMSC threshold, receiver operator characteristic (ROC) analysis determined the sORP cutoff associated with the highest predictive values. TMSC was significantly negatively correlated with sORP across all participants ($r = 0.86$, $P < 0.001$), among infertile patients ($r = 0.729$, $P < 0.001$), and among fertile controls ($r = 0.53$, $P < 0.001$). A 20-million TMSC threshold determined an sORP cutoff value of 2.34 mV/10^6 sperm/ml to be associated with 82.9% sensitivity, 82.8% specificity, 91.5% positive predictive value (PPV), 68.5% negative predictive value (NPV), and 82.9% overall accuracy. Compared with previously reported cutoff values in searched literature, the 2.34 mV/10^6 sperm/ml cutoff value identified in our study yielded the highest overall diagnostic accuracy in the evaluation of infertile men.

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INTRODUCTION

Infertility is a public health problem that affects approximately 48.5 million couples globally, with 50% of the cases attributed to a male factor.[1] Clinicians mainly rely on conventional methods, such as semen analysis, to assess a man's fertilizing ability.[2] However, a shortcoming of this approach is that it may underestimate the "true" male fertility potential as it does not assess all the sperm functions.[2] Moreover, the predictive ability of semen analysis is relatively poor, particularly because of the variability in individuals' semen parameters.[3] Therefore, the development of advanced sperm function tests to assess sperm quality and function is required in order to assess male fertility potential more accurately and to detect possible etiologies of male infertility. Examples of such tests include measures of oxidative stress (OS) and sperm DNA fragmentation (SDF), which are increasingly being used as diagnostic parameters in the recent years.[4,5]

OS, a central cause of male infertility,[6,7] results from an imbalance between oxidants and reductants that originate from either an increased generation of reactive oxygen species (ROS) or decreased seminal antioxidants. About 30%–40% of infertile men have elevated ROS levels in their seminal plasma.[7] While optimal sperm functions, for example, motility, capacitation, hyperactivity, and acrosome reaction[8,9] require moderate physiological ROS levels, elevated OS levels may have a detrimental impact on sperm-fertilizing ability.[7,10] Hence, OS measurement has been used as a biomarker to assess semen quality and as a complementary test to routine semen analysis.

Previous OS assessment methods included the measurement of single features of the redox system such as oxidants (ROS in semen via chemiluminescence assays) or antioxidants (total antioxidant capacity in seminal plasma via colorimetric assays), lipid peroxidation via thiobarbituric acid assay or 4-hydroxynonenal,[11,12] apoptotic markers,[13] and OS-modified protein alterations by proteomic tools.[14,15] While such traditional OS measurement methods are useful, they are outdated, time sensitive, time consuming, and tedious and require large sample volumes or special technical skills.[16] A more accurate OS measure is

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the static oxidation-reduction potential (sORP), which provides an overview of the redox system by assessing the net balance between oxidants and reductants in any given medium. Recently, sORP in semen has been easily and comprehensively measured by the MiOXSYS system, a new technology that enables the wider application of OS analysis in clinical and research settings.[17] sORP results provided by MiOXSYS are standardized, reliable, and reproducible, compared to previous ROS assays.[6]

Attempts to enhance the predictive power of conventional semen analysis sought to identify the individual semen parameter that could accurately assess male fertility potential. Recent studies have suggested total motile sperm count (TMSC) as one of the most important semen analysis parameters that assesses the male infertility severity and provides vital information that might influence treatment decisions.[18–20] There is a stronger correlation between TMSC and pregnancy rate (both natural and assisted) than with the WHO 2010 classification system cutoff values.[18] In addition, compared to the WHO 2010 cutoff values, TMSC has a prognostic value in the prediction of total fertilization failure in couples undergoing intrauterine insemination (IUI) or *in vitro* fertilization (IVF).[21–23] Moreover, TMSC has a higher predictive value for laboratory results and pregnancy outcomes among individuals undergoing intracytoplasmic sperm injection (ICSI) secondary to male infertility.[24]

Although sORP has been correlated with different semen parameters such as concentration, motility, and morphology,[7] to the best of our knowledge, there are no published data exploring the correlation between TSMC and sORP, or identifying the sORP cutoff and its objective measures of test performance based on the TMSC result. Therefore, among infertile men attending our infertility unit and fertile controls, the current study aimed to: (i) assess the correlation between sORP and TMSC; (ii) identify the sORP cutoff value that reliably predicts fecundity based on the TMSC result; and (iii) search the published literature and compare the diagnostic accuracy of our sORP cutoff value with those of previous studies.

PATIENTS AND METHODS

Ethics, design, and sample

This retrospective chart review study was approved by our medical research center (Institutional Review Board, Protocol #01-17-186), and a waiver of signed informed consent was used. We searched databases and retrieved the medical records of men with primary or secondary infertility attending our infertility unit at Hamad Medical Corporation over a period of 12 months (January 12, 2015 to January 12, 2016). Exclusion criteria included those with any medical condition that may affect the oxidative status, for example, present or past history of pyospermia/azoospermia, history of testicular injury/infection, varicocele, and vasal reconstruction, in addition to habitual/occupational activities linked with a potentially higher OS (*e.g.*, drinking >2 alcoholic beverages per week, or excessive exposure to radiation/chemicals). Patients under specific medications (ketotifen or nonsteroidal anti-inflammatories) and those with a history of chronic disease (*e.g.*, chronic lung diseases and chronic renal/liver failure) were also excluded. Of the 3142 potential participants, 1168 patients met the inclusion criteria and agreed to participate in the study (the infertile group) (**Supplementary Figure 1**). The control group comprised data of 100 fertile men, recruited through an advertisement at our institution, who provided proof of pregnancy in the past 2 years (child's birth certificate and medical report from the spouse's gynecologist ascertaining that the pregnancy was spontaneous). For both infertile patients and fertile controls, demographic data, conventional semen analysis, and advanced sperm function test results (sORP and SDF) were retrieved.

Assessment of conventional semen parameters

Each semen sample was assessed for macroscopic parameters including color, pH, ejaculate volume, age of the sample, and viscosity after complete liquefaction. Based on the WHO 5th edition guidelines,[25] an aliquot of the sample was examined for sperm concentration, total sperm count, total and progressive motility, as well as sperm morphology. The samples were analyzed manually using an hemocytometer. Sperm motility was evaluated and categorized as progressive or nonprogressive. Morphological evaluation was performed using the Diff–Quik staining protocol, and 4% normal morphology was used as a cutoff based on strict criteria.[25] TMSC was calculated using the following formula: semen volume (ml) × sperm concentration (million ml^{-1}) × total motility (%)/100. Normal semen analysis was defined as sperm concentration of ≥15 million ml^{-1} and total sperm motility ≥40% and normal sperm morphology ≥4%; if one or more of these three criteria were unfulfilled, the semen analysis was defined as abnormal.

Assessment of ORP

Oxidative stress was measured by sORP in a 30 µl aliquot of liquefied semen by using a new MiOXSYS™ galvanostatic technology (Aytu Bioscience, Inc., Englewood, NJ, USA). This system comprises the insertion of a semen-filled disposable sensor into the analyzer to measure the electron transfer from reductants (antioxidants) to oxidants using a steady low-voltage reducing current, thus reflecting aggregate measures of the current oxidant and antioxidant activity in the sample. Higher sORP levels indicate an imbalance in the activity of all available oxidants relative to all available antioxidants in the seminal ejaculate, leading to a state of OS. sORP values were divided by the sperm concentration (×10^6 ml^{-1}) and represented as mV/10^6 sperm/ml in order to control for differences in sperm number.[10]

Assessment of sperm DNA fragmentation

SDF was measured using the Halosperm kit (Halotech DNA, S.L., Madrid, Spain) according to manufacturer instructions, based on the sperm chromatin dispersion test.[26] SDF level cutoff taken as high was ≥30%. As this test is not routinely used for the assessment of male infertility, data were available for only 309 infertile patients but for all the fertile controls.

Statistical analyses

SPSS version 20 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses, with significance set at $P < 0.05$. The fertile and infertile groups were compared. Quantitative variables were presented using median (interquartile range), and Wilcoxon's rank-sum test compared the quantitative variables, for example, age, abstinence, volume, sperm count, sperm morphology, TMSC, and sORP. Spearman's correlation assessed the relationship between sORP and TMSC. With a 20-million TMSC threshold[18] across our sample, receiver operator characteristic (ROC) analysis determined the sORP cutoff associated with the highest objective values of test performance.

Literature search

A literature search was conducted using PUBMED and MEDLINE databases looking for original articles utilizing the MiOXSYS technology to examine the sORP of infertile men. The search was executed using the following keywords: "oxidation reduction potential," "semen parameters," and "male infertility," and by the articles published during the past 5 years (sORP is a recent phenomenon) in English

language. Only articles identifying sORP cutoff values were retrieved, and the search yielded four studies.[2,10,27,28]

In order to evaluate the diagnostic accuracy of our generated cutoff, we compared its test performance results with those reported in the literature. Finally, we examined whether the application of cutoff values from previous studies[2,10,27,28] to our data would result in any subsequent changes to our initial diagnostic accuracy indices.

RESULTS

Infertile patients were roughly 3 years older than fertile controls (**Table 1**). Sperm count, total motility, progressive motility, normal morphology, and TMSC were significantly lower among the infertile patients compared to those in the fertile controls ($P \leq 0.001$). Conversely, SDF and sORP were significantly higher among the infertile compared to those in the fertile group ($P \leq 0.001$).

Table 2 depicts the correlation between sORP and a range of sperm parameters for all participants in the study and separately for infertile patients and fertile controls. For all participants, there was a significant strong negative correlation between sORP and each of TMSC, sperm count, total and progressive motility, and normal morphology. Conversely, a significant positive correlation was observed between sORP and SDF. Across both infertile patients and fertile controls, sORP and TMSC were significantly negatively correlated ($P \leq 0.001$; **Figure 1**).

Figure 2 shows the ROC curve analysis between sORP and TMSC. ROC curve analysis obtained an sORP cutoff value of 2.34 mV/10^6 sperm/ml, which was associated with 82.9% sensitivity, 82.8% specificity, 68.5% negative predictive value (NPV), 91.5% positive predictive value (PPV), and 82.9% overall accuracy (area under the curve = 0.9).

In order to compare our cutoff values and diagnostic accuracy with the literature, **Table 3** demonstrates sORP cutoff values and four objective measures of test performance from previous research compared with those of the current study. Our observed sORP cutoff value (2.34 mV/10^6 sperm/ml, based on a TMSC) was higher than that of previous studies and generated higher sensitivity and overall accuracy than those reported by other published sORP cutoff values (based on fertility outcome or semen parameter status). Our 2.34 mV/10^6 sperm/ml cutoff value also greatly reduced the false-negative rates previously reported by all the four published studies, and moderately reduced the false-positive rates previously reported by two of the four published studies.[2,10,27,28]

Table 4 demonstrates the changes in the objective measures of test performance after applying the cutoff values of previous studies to our data. Overall, our identified sORP cutoff (using TMSC) generated indices that were better in 18/25 instances, worse in 5/25 instances, and similar in 2/25 instances. This highly suggested the validity of using an sORP value that is based on the TMSC during the evaluation of male fertility.

DISCUSSION

We assessed semen parameters and results of advanced sperm function tests among infertile patients and fertile controls. Our results revealed significantly lower sperm concentration, total and progressive motility, sperm morphology, and TMSC in infertile men compared to fertile men ($P \leq 0.001$). Conversely, significantly higher sORP and SDF were detected among the infertile men compared to the fertile controls ($P \leq 0.001$).

Conventional semen analysis methods have long been criticized by their poor ability to predict conception accurately.[3,25,29–31] Conversely, sperm quality is probably more precisely expressed with TMSC which is derived by combining three different parameters (semen volume, sperm concentration, and motility) to yield a better indicator. Research revealed a stronger correlation between TMSC

Table 1: Selected demographic and sperm data of infertile patients and fertile controls

Parameter	*Infertile patients (n=1168), median (IR)*	*Fertile controls (n=100), median (IR)*	*P*
Age (year)	35 (31–40)	32 (27–35)	<0.001
Abstinence (day)	3 (3–4)	3 (3–4)	0.08
Volume (ml)	3 (2–4)	3 (2–3.4)	0.06
Concentration (million per ml)	26 (11–48)	55 (40–74)	<0.001
Total motility (%)	55 (40–63)	62 (53.5–67.8)	<0.001
Progressive motility (%)	10 (0–20)	32 (21.3–33)	0.001
Morphology (%)	4 (2–6)	9 (6–13)	<0.001
TMSC (million per ejaculate)	37.1 (11.9–77.9)	83 (52.8–129)	<0.001
SDF* (%)	23 (14.3–34)	15 (11–19)	<0.001
sORP (mV/10^6 sperm/ml)	1.8 (0.9–4.3)	0.9 (0.7–1.4)	<0.001

*For this test, the sample comprised 309 infertile patients and 100 fertile controls individuals. IR: interquartile range; TMSC: total motile sperm count; SDF: sperm DNA fragmentation; sORP: static oxidation reduction potential

Table 2: Correlation between oxidation reduction potential and sperm parameters for whole sample, infertile patients, and fertile controls

Parameter	*Whole sample (n=1268)*		*Infertile patients (n=1168)*		*Fertile controls (n=100)*	
	sORP	*P*	*sORP*	*P*	*sORP*	*P*
Age (year)	0.01	0.7	–0.15	0.612	0.03	0.97
Volume (ml)	0.05	0.08	0.04	0.168	–0.037	0.71
Concentration (million per ml)	–0.725	<0.001	–0.866	<0.001	–0.804	<0.001
Total motility (%)	–0.38	<0.001	–0.38	<0.001	–0.118	0.243
Progressive motility (%)	–0.419	<0.001	–0.497	<0.001	–0.322	<0.001
Morphology (%)	–0.57	<0.001	–0.562	<0.001	–0.195	0.052
TMSC (million per ejaculate)	–0.86	<0.001	–0.729	<0.001	–0.530	<0.001
SDF (%)	0.258	<0.001	0.222	<0.001	0.004	0.978

Spearman's test. TMSC: total motile sperm count; SDF: sperm DNA fragmentation; sORP: static oxidation reduction potential

Table 3: Comparison between the predictive power of various static oxidation reduction potential cutoff values

Study	*Patients (n)*	*Cutoff value (mV/10^6 sperm/ml)*	*Outcome measures*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*Overall accuracy (%)*	*False negative (%)*	*False positive (%)*
Previous studies										
Agarwal *et al.*[27]	695	1.42	Fertile/infertile men	60.6	74.3	93.3	24.3	62.6	33.8	23.7
Arafa *et al.*[28]	365	1.38	Normal/abnormal semen	63.3	87.8	97.6	23.2	66	32.8	1.6
	415	1.41	Fertile/infertile men	57.3	78	95	20	60	37.8	1.6
Agarwal *et al.*[2]	157	1.36	Normal/abnormal semen	69.6	83.1	85.3	65.9	75.2	17.8	7
Agarwal *et al.*[10]	59	1.48	Normal/abnormal semen	60	75	45	84.6	71.2	10	18.6
Current study	1268	2.34	>20/<20 million TMSC	82.9	82.8	91.5	68.5	82.9	5.3	11.9

PPV: positive predictive value; NPV: negative predictive value; TMSC: total motile sperm count

Table 4: Applying cutoff values of previous studies* to our data: subsequent changes in predictive power

Comparison	*Cutoff value (mV/10^6 sperm/ml)*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*Overall accuracy (%)*
Current study versus Agarwal *et al.*[27]	1.42	↓↓	↓	↑	↓↓↓	↓↓
Current study versus Arafa *et al.*[28]	1.38	↔	↓↓	↓↓	↑	↓
Current study versus Arafa *et al.*[28]	1.41	↓↓	↓	↑	↓↓↓	↓↓
Current study versus Agarwal *et al.*[2]	1.36	↔	↓↓	↓↓	↑	↓
Current study versus Agarwal *et al.*[10]	1.48	↓	↓↓	↓↓	↑	↓

*Using the original outcome measures employed in the given studies. PPV: positive predictive value; NPV: negative predictive value; ↔ nil or minimal (0–5%) change in value; ↓: decrease in value >5%–10%; ↓↓: decrease in value >10%–20%; ↓↓↓: decrease in value >20%; ↑: increase in value 5%–10%

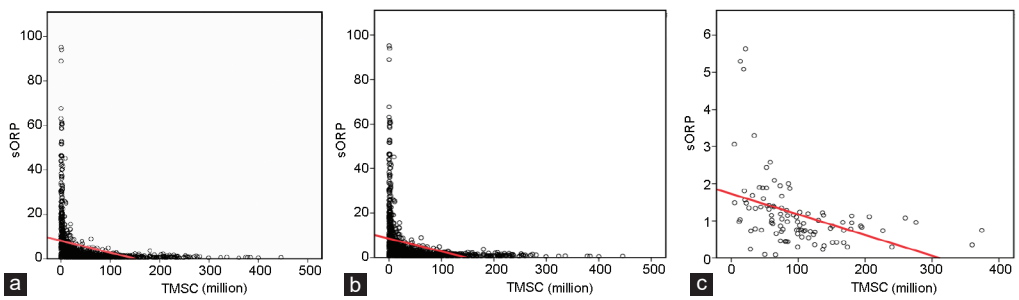

Figure 1: Correlations between sORP and TMSC in the (**a**) whole sample, (**b**) infertile patients, and (**c**) fertile controls. sORP: static oxidation reduction potential; TMSC: total motile sperm count.

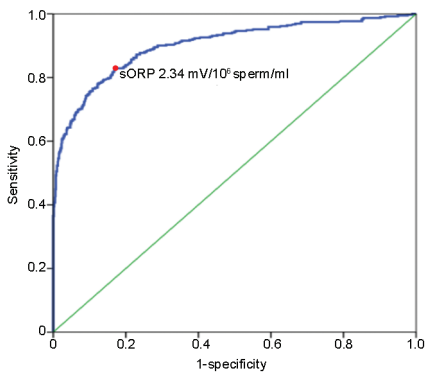

Figure 2: Identifying the ORP cutoff value: ROC curve analysis for sORP against TMSC. sORP: static oxidation reduction potential; ROC: receiver operator characteristic; TMSC: total motile sperm count; ORP: oxidation reduction potential.

and spontaneous ongoing pregnancy rate than the cutoff values of WHO 2010 classification system.[18] Three prognostic groups of semen quality have been proposed according to the TMSC result: (i) $<5 \times 10^6$, (ii) $(5–20) \times 10^6$, and (iii) $>20 \times 10^6$ spermatozoa (the latter being considered a normal TMSC value).[18] The authors recommended assisted conception when the TMSC is $<20 \times 10^6$ spermatozoa; IVF or ICSI for a TMSC $<5 \times 10^6$; and IUI for a TMSC value $(5–20) \times 10^6$.[19] In our study, TMSC and total motility were significantly lower in infertile men compared with fertile controls ($P \leq 0.001$).

Advanced sperm function tests are progressively utilized in the evaluation of infertile men, being a better representation of the true fertility potential.[4,32] OS has been considered a common pathway through which several etiologies can impair sperm production. Sperm are particularly vulnerable to OS as their membranes are rich in polyunsaturated fatty acids and their scant cytoplasm lacks an efficient enzymatic antioxidant system. Consequently, OS results in lipid peroxidation, DNA fragmentation, and abortive apoptosis,[13,33] which all can alter sperm production. Moreover, significant negative correlations have been detected between OS and semen parameters, fertilization rate, embryonic development, and pregnancy rate.[34]

Our results revealed significant negative correlations between sORP and TMSC, sORP and sperm count, sORP and progressive motility, and sORP and total motility in the whole semen samples and also in the infertile and fertile groups. An interesting point was that, across both study groups (infertile patients and fertile controls), sORP and TMSC were significantly negatively correlated ($P \leq 0.001$), suggesting that this correlation remains maintained regardless of the TMSC levels and the fertility status, albeit the correlation was stronger in infertile patients. In terms of SDF, high levels of DNA damage may have negative consequences on fertility by decreasing the chances of fertilization, pregnancy, early embryo development, and implantation.[13]

Our findings revealed that both sORP and SDF were significantly higher in infertile men compared to fertile men in support of others.[4,35] Furthermore, a significant, but weak, positive correlation between sORP and SDF was detected in the whole sample and in infertile men, which is also in congruence with prior reports.[36] This significance was not detected in the fertile controls, which could be explained by the fewer SDF measures that were performed in this study group.

Our ROC curve analysis determined the best sORP cutoff value that accurately predicts sperm quality, based on a TMSC threshold of 20 million. Our results revealed a value of 2.34 mV/10^6 sperm/ml to be associated with good objective measures of test performance, exhibiting 82.9% sensitivity, 82.8% specificity, 68.5% NPV, 91.5% PPV, and 82.9% overall accuracy (area under the curve = 0.9). Earlier studies utilizing MiOXSYS assessment of sORP levels reported lower cutoff values (**Table 3**); however, they were not based on the TMSC variable, rather they were based on fertility outcome (fertile/infertile) or semen parameter status (normal/abnormal). Values of 1.48 mV/10^6 sperm/ml and 1.38 mV/10^6 sperm/ml were reported in differentiating normal from abnormal semen (defined by the presence of ≥1 abnormalities in sperm parameters).[10,28] A value of 1.36 mV/10^6 sperm/ml was the cutoff detected to differentiate infertile men from a fertile control.[2] A recent multicenter study using a standardized MiOXSYS approach to determine the validity of sOPR testing among 594 infertile men and 101 fertile controls determined a cutoff value of 1.42 mV/10^6 sperm/ml to be associated with 60.6% sensitivity, 74.3% specificity, 93.3% PPV, 24.3% NPV, and 62.6% overall accuracy.[27] Such similar results reported from different centers underscore the validity and reproducibility of sORP testing as a valuable tool in the evaluation of infertile men.

Despite the lower cutoff values reported in the aforementioned studies, we observed a higher sORP diagnostic accuracy level using TMSC as an outcome measure. Particularly, the false-negative rate was reduced (up to 32.5%) compared to previous studies, although the false-positive rate was reduced only compared to two studies,[10,27] but higher than the other two remaining studies[10,28] probably because these studies had a considerably lower sample size with uneven fertile/infertile groups and normal/abnormal semen groups.

In order to make more sense of our findings, we compared our objective measures of test performance (using 2.34 mV/10^6 sperm/ml cutoff) to those obtained after we applied cutoff values from previous studies (using their original outcome measures) to our data (**Table 4**). Overall, our identified sORP cutoff (using TMSC) generated indices that were better in 18/25 instances, worse in 5/25 instances, and similar in 2/25 instances. This highly suggests the validity of using an sORP value that is based on the TMSC during the evaluation of male fertility.

A diagnostic test is considered valid for clinical use if it has a combination of high sensitivity, specificity, and accuracy as well as negative and positive predictive values. Hence, our preferred sORP cutoff of 2.34 mV/10^6 sperm/ml with its corresponding high sensitivity and predictive value is more preferably used to identify OS among patients at risk of infertility in the clinical setting. In addition to its ability to provide an assessment of the patient's redox potential, an sORP value of ≤2.34 mV/10^6 sperm/ml can forecast a favorable TMSC which is believed to be the single most important semen parameter capable of predicting fecundity.

One limitation of our study is that the samples were collected from only one medical center in Qatar; hence, there is a need to conduct similar studies in multiple centers from different regions around the world to validate our findings. Another limitation would be the age difference between the study groups which although small, was statistically significant. Furthermore, there is a gross difference between the sizes of both study groups; however, this is attributed to the difficulty in recruiting normal fertile controls for research purposes only.

CONCLUSION

OS is a major indicator of male infertility and can be accurately and easily assessed by measuring the sORP in semen samples using the MiOXSYS system. TMSC is an essential parameter to evaluate during assessment of the severity of male infertility. Our findings show a significant negative strong correlation between sORP and TMSC, highlighting its potential use as a predictor of fertility. Moreover, based on total motile sperm count, this study established a new diagnostic sORP cutoff (2.34 mV/10^6 sperm/ml) that generated higher sensitivity and overall accuracy than those reported by other published sORP cutoffs.

AUTHOR CONTRIBUTIONS

AA, SAS and HE supervised the entire study, including the procedures, conception, design, and completion. MM was responsible for the collection of data. AM contributed to the data analysis and drafted the article. MA and WEA participated in the interpretation of the study data and in revisions to the article. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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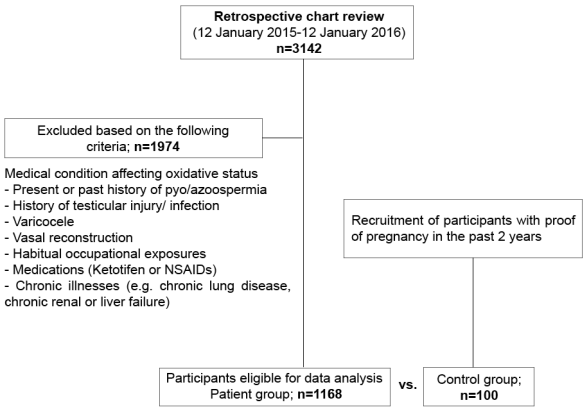

Supplementary Figure 1: Study flow diagram.