Correlation of oxidation–reduction potential with hormones, semen parameters and testicular volume

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Original Article

Seminal oxidative stress (OS) is a major cause of male factor infertility and can be measured as oxidation–reduction potential (ORP). Studies showed significant negative relationships of ORP with sperm count, motility or DNA integrity. Since these parameters are also positively or negatively associated with reproductive hormones follicle-stimulating hormone (FSH), luteinising hormone (LH), testosterone, testicular volume and the occurrence of varicocele, it is important to understand the mechanistic relationship between ORP and hormonal and/or testicular parameters. Therefore, we studied the relationship between ORP levels, standard hormone profiles and testicular volume in infertile men with and without varicocele. Results show a highly significant negative relationship of ORP with testicular volume and significantly positive correlations with FSH and LH. Yet, when adding varicocele as covariate, the relationship with FSH/LH became nonsignificant. Contrary, the presence of varicocele had only a contributing influence on the association of ORP with the testis volume. No association was found with estradiol. We propose that since OS causes degeneration of Sertoli cell with testicular shrinkage, such negative effect would result in a negative feedback on the hypothalamus with less inhibin secretion. This may result in increased secretion of LH and FSH. Thus, systemic and/or local OS may be responsible for smaller testis volumes.

Keywords
follicle-stimulating hormone, luteinising hormone, oxidation–reduction potential, oxidative stress, testicular volume

1 | Introduction

According to the World Health Organization (WHO), measuring oxidative stress is critically important in the assessment of male infertility (World Health Organization (WHO) (2010)). Oxidative stress results from an overload of reactive oxygen species (ROS) and/or a deficiency of antioxidants (Hampl, Drabkova, Kandar, & Stepan, 2012). Reactive oxygen species are chemically highly reactive molecules that are normally scavenged and deactivated by antioxidants to maintain a physiological balance. Nonetheless, low levels of ROS are required for key physiological steps of fertilisation, including capacitation, hyperactivation, acrosome reaction and spermatozoa–oocyte binding (Aitken, Irvine, & Wu, 1991; Lamirande & Cagnon, 1993). However, in states of oxidative stress, excessive levels of ROS potentially inhibit these key steps of the fertilisation process along with implantation and embryonic development (Lamirande & Lamothe, 2009). Reactive oxygen species are also a cause of birth defects or miscarriage (Mates, Segura, Alonso, & Marquez, 2012) and exert their effects on fertility by negatively impacting spermatozoa through lipid peroxidation, DNA fragmentation and apoptosis (Sawyer, Mercer, Wiklendt, & Aitken, 2003).
Spermatozoa are highly susceptible to oxidative stress because they have an extraordinary high concentration of polyunsaturated fatty acids in their plasma membrane, making them vulnerable to lipid peroxidation (Sanocka & Kurpisz, 2004). They also lack a well-developed, protective antioxidant system due to their low amount of cytoplasm (Gomez et al., 1996). In addition, spermatozoa are exposed to intrinsic and extrinsic sources of ROS. Intrinsic sources include Sertoli cells, leucocytes in seminal fluid, residual cytoplasm of morphologically abnormal spermatozoa and pathological conditions, including varicocele (Hipler, Gornig, Hipler, Romer, & Schreiber, 2000; Ko, Sabanegh, & Agarwal, 2014; Shiraishi, Matsuyama, & Takihara, 2012). Extrinsic sources include smoking, alcohol consumption and radiation exposure (Lavranos, Balla, Tzortzopoulou, Syriou, & Angelopoulou, 2012).

The latest, most efficient mode of assessing seminal oxidative stress is the measurement of oxidation-reduction potential (ORP) by means of the MiOXSYS Analyzer (Agarwal, Roychoudhury, Bjugstad, and Cho (2016)). Oxidation-reduction potential reflects the redox balance between oxidants and antioxidants, specifically measuring the transfer of electrons from antioxidants to oxidants (McCord, 2000). Oxidation-reduction potential measurement is superior to pre-established modes of assessment, including the chemiluminescent detection of reactive oxygen species, total antioxidant capacity and determination of malondialdehyde (Agarwal, Roychoudhury et al., 2017). Hence, it provides a complete, comprehensive measurement of all known and unknown oxidants and antioxidants in a semen sample without relying on a single marker of oxidative stress (Agarwal & Wang, 2017). Moreover, measurement of ORP is less time-consuming, less expensive, and utilises a smaller seminal sample volume (Agarwal, Roychoudhury et al., 2016).

Various studies have investigated the potential diagnostic role of ORP measurement in male infertility assessment (Agarwal, Sharma, Roychoudhury, Plessis, & Sabanegh, 2016; Arafa et al., 2018; Roychoudhury et al., 2003). Studies on infertile males have shown that ORP negatively correlates with semen parameters, including sperm concentration and motility (Agarwal, Roychoudhury et al., 2017; Agarwal & Wang, 2017). In addition, males afflicted with infertility, oligoasthenozoospermia and varicocele display higher ORP levels in comparison with their normal controls (Agarwal, Roychoudhury et al., 2017; Agarwal & Wang, 2017). After all, high ORP levels reflect oxidative stress. An ORP cut-off value for fertility and various sperm parameters of 1.36 mV/10^6 sperm/ml has also been established (Agarwal, Roychoudhury et al., 2017). However, there are no studies so far investigating the relationship between the seminal ORP levels, reproductive hormones and testicular volume. This is important as these parameters are negatively correlated with sperm count, motility and sperm functional parameters. Therefore, identifying the potential relationship and impact of ORP and seminal oxidative stress on hormone levels and testicular volume will enhance our understanding of oxidative stress and the diagnostic use of ORP assessment in male infertility.

We evaluated the seminal ORP levels to determine its relationship with standard hormone profiles and testicular volumes in men attending an andrology clinic for fertility problems.

2 | MATERIALS AND METHODS

2.1 | Patients

The study was approved by the Institutional Review Board of the Medical Research Center of the institute. A waiver of informed consent was obtained prior to data collection.

This was a retrospective study of 660 patients attending the male infertility unit at the Department of Urology, Hamad Medical Corporation, Doha, Qatar, between January and March 2017. Patients between the ages of 18 and 70 years complaining of infertility were included in the study. In addition, the presence of unilateral or bilateral varicocele was recorded. The exclusion criteria, on the other hand, involved patients with documented genetic abnormalities, testicular cancers, genitourinary tract infections or those receiving hormone replacement or antioxidant therapy.

2.1.1 | Data collection

The medical records were searched to collect demographic and clinical data and laboratory investigations for patients.

2.1.2 | Semen analysis

Semen analysis was performed after 3–5 days of sexual abstinence. Collection was done through masturbation into a clean plastic container. Samples were incubated at 37°C and allowed to liquefy for 30 min before analysis. The analysis was performed according to WHO Fifth Edition guidelines adopted in 2010.

2.1.3 | Sperm DNA fragmentation

Sperm DNA fragmentation (SDF) was measured using the Halosperm G2 Test kit. This kit determines the degree of DNA damage of human spermatozoa through a process called sperm chromatin dispersion (SCD), a process, which involves the denaturation and controlled lysis of the sample in an appropriate medium. Spermatozoa with intact DNA produce a dispersion halo as a result of the chromatin released from nuclear proteins that can be easily analysed using fluorescence or bright field microscopy. In contrast, spermatozoa with fragmented DNA will not produce this halo. A cut-off of ≤30% DNA damage was considered negative (Fernandez et al., 2005).

2.1.4 | Measurement of oxidation-reduction potential

The galvanostat-based technology MiOXSYS system (Aytu BioScience, Englewood, CO) was used to measure ORP. A 30 µl aliquot of the liquefied semen sample was applied to a pre-inserted
sensor at room temperature. The test starts once the sample fills the reference electrode closing the electrochemical circuit. After 4 min, the ORP value in millivolts (mV) was displayed on the screen. ORP values were then normalised according to the seminal sperm concentration and expressed as mV/10⁶ sperm/ml (Agarwal, Sharma et al., 2016).

2.1.5 | Hormone analysis

Blood samples for hormonal assay were collected from each patient individually between 7:00 a.m. and 9:00 a.m., and the analysis was done in the endocrine laboratory of Hamad Medical Center using the immunoassay chemiluminescence method, Architect i1000SR® (Abbott Systems, Illinois, USA). The hormonal profile included follicular-stimulating hormone (FSH) (n = 1–19 IU/L), luteinising hormone (LH) (n = 1–9 IU/L), prolactin (n = 73–407 mIU/L), total testosterone (n = 10.4–35 nmol/L) and estradiol (n = 73–275 pmol/L).

2.1.6 | Testicular volume

Testicular volume measurements were performed using scrotal ultrasonography. These scans were carried out by a consultant radiologist, using a 7.5 MHz probe and were performed using the length (longitudinal diameter), width (transverse diameter) and height (anterior–posterior diameter) of the testes. The testicular volume was calculated using (a) the formula for an ellipsoid (formula: length (L) × width (W) × height (H) × 0.52.

2.2 | Statistical analysis

Statistical analysis was performed using MedCalc statistical software (V. 18.6; MedCalc Software bvba, Ostend, Belgium). After checking for normal distribution of the data by means of the chi-squared test, non-parametric tests (Spearman rank correlation, Mann–Whitney, Kruskal–Wallis and Jonckheere–Terpstra test for trend analysis) were applied. Statistical significance was defined as p < 0.05.

For further statistical analysis, patients were categorised according to FSH level (low: <1.0 IU/L; normal: 1.0–18.0 IU/L; high: >18.0 IU/L), LH level (low: <1.8 IU/L; normal: 1.8–8.6 IU/L; high: >8.6 IU/L) and normal testis volume (small: <18 ml; normal: ≥18 ml; Bahk, Jung, Jin, & Min, 2010; Tijani, Oyende, Awosanya, Ojewola, & Yusuf, 2014).

3 | RESULTS

A total of 660 patients between 18 and 73 years of age were included in the study; the mean age was 35.78 ± 7.63 years. Of these 660 patients, 481 showed no varicocele, 102 unilateral and 77 bilateral varicocele. Table 1 depicts a summary of study results. As expected, a weak, but significant negative correlation (r = −0.0991, p = 0.0439) between the serum testosterone concentration and the varicocele status was seen with the difference in testosterone levels between patients with and without varicocele (mean ± SD: 16.67 ± 7.04 nmol/L and 23.65 ± 75.63 nmol/L, respectively) was significant (p = 0.0347).

Table 2 shows the categorisation of subjects according to FSH (low: <1.0 IU/L; normal: 1.0–18.0 IU/L; high: >18.0 IU/L) and LH levels (low: <1.8 IU/L; normal: 1.8–8.6 IU/L; high: >8.6 IU/L) as well as normal testis volume (low: <18 ml; normal: ≥18 ml). FSH and LH levels were measured in a total of 418 patients. Approximately 79% of the patients had low levels of FSH (mean ± SD: 2.84 ± 1.35 IU/L). Normal FSH levels were found in 11% of the patients (mean ± SD: 6.94 ± 1.21), while 9.8% exhibited high levels (mean ± SD: 24.33 ± 36.83). For LH, approximately 86% of the patients had normal LH levels (mean ± SD: 3.93 ± 1.45), while 7.1% and 6.6% of subjects had low (mean ± SD: 3.93 ± 1.45) and high (mean ± SD: 13.48 ± 10.45) LH levels respectively. The testicular volume was assessed in 199 patients with 51.7% of the patients having normal testicular volume (mean ± SD: 22.71 ± 3.56 ml), while the rest had low testicular volume (mean ± SD: 14.11 ± 2.64 ml).

A highly significant negative correlation was detected between ORP and sperm count (r = −0.793, p < 0.001), motile sperm count (r = −0.579, p < 0.001), progressive motility (r = −0.431, p < 0.001) and normal sperm morphology (r = −0.458, p < 0.001). Contrary, ORP levels were weakly positively correlated with sperm DNA fragmentation (r = 0.264), yet significantly (p < 0.001). Oxidation–reduction potential was also significantly correlated with testicular volume (r = −0.386; p < 0.0001; Supporting information Figure S1).

Oxidation–reduction potential correlated significantly positively with serum hormone levels, FSH (r = 0.273; p < 0.0001) and LH levels (r = 0.182; p = 0.0002), while there was no correlation with estradiol (r = 0.0701; p = 0.1689) and prolactin (r = 0.0660; p = 0.1791). However, when taking the varicocele status of the patients as covariate into account, the relationships between ORP and FSH (r = 0.2026; p = 0.6804) and LH (r = 0.01075; p = 0.8270), respectively, were not significant. Figures 1 and 2 show the relationship between serum FSH levels and its influence by the varicocele status. In contrast, the varicocele status had no significant influence on the relationship between ORP and the testicular volume (r = −0.2934; p < 0.0001) as high ORP values were only observed in the group of patients with small testis volume and bilateral varicoceles (Figure 3).

While serum FSH and LH concentrations showed significant negative relationships with the volume of the testes (r = −0.516; p < 0.0001 and r = −0.412; p < 0.0001, respectively), no correlation was found between testicular volume and serum testosterone (r = −0.0127; p = 0.8668) and estradiol concentrations (r = −0.0750; p = 0.3398). There was a weak, but significant negative relationship between testicular volume and serum prolactin concentration (r = −0.198; p = 0.0081). In addition, testicular volume correlated significantly and positively with sperm count (r = 0.360; p < 0.0001) and normal sperm morphology (r = 0.163; p = 0.0217). No relationship was found between testicular volume and motility (r = −0.0737; p = 0.3008).
Discussion

Oxidation-reduction potential was negatively correlated with various sperm parameters as shown previously (Agarwal, Roychoudhury et al., 2017), specifically, as ORP levels rise, seminal sperm count, motility and normal sperm morphology decline, supporting the diagnostic use of ORP assessment in male infertility. Moreover, results of this study illustrate a negative correlation between ORP and testicular volume. Testicular volume depends on Sertoli cell mass since Sertoli cells line up seminiferous tubules and make up to 90% of the testicular tissue (Stewart et al., 2009). The negative relationship between oxidative stress as measured by ORP and testicular volume might reflect a primary causative relationship as it appears to be independent from the presence of varicocele. Perhaps, systemic and/or local oxidative stress might be the cause of this negative relationship by reducing the volume and number of Sertoli cells via lipid peroxidation, DNA fragmentation and apoptosis (Plymate, Paulsen, & McLachlan, 1992), which would have a negative feedback on the hypothalamus leading to increased LH and FSH secretion. Yet, the serum levels of these hormones appear

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low (&lt;1.0 IU/L)</th>
<th>Normal (1.0 to 18.0 IU/L)</th>
<th>High (&gt;18.0 IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>331</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.84 ± 1.35</td>
<td>6.94 ± 1.21</td>
<td>24.33 ± 36.83</td>
</tr>
<tr>
<td>LH</td>
<td>30</td>
<td>362</td>
<td>26</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.42 ± 0.35</td>
<td>3.93 ± 1.45</td>
<td>13.48 ± 10.45</td>
</tr>
<tr>
<td>Testis volume</td>
<td>96</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>14.11 ± 2.64</td>
<td>22.71 ± 3.46</td>
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not to be affected by ORP as these correlations were not significant if the varicocele status was added as covariate. In fact, testicular degeneration, reflected by a decrease in Sertoli cell number, has been histologically detected in animal testicular tissue induced with oxidative stress (Boujbiha et al., 2009). In human testicular tissue, a reduction in seminiferous tubule diameter and testicular atrophy was detected in males affected by varicocele with decreased numbers of testosterone-positive cells (Sirvent et al., 1990). In fact, lower serum testosterone levels in varicocele patients have been shown for decades (Ando et al., 1984; Tanrikut et al., 2011); the varicocelectomy seems to reverse low testosterone secretion, at least in hypogonadal men (Abdel-Meguid et al., 2014). Varicocele is a known cause of oxidative stress, and patients with varicocele have higher ORP levels than fertile controls (Agarwal, Wang et al., 2017). The varicocele-related oxidative stress will then add and exacerbate the problem.

If oxidative stress plays a role in reducing testicular volume via Sertoli cell degeneration, it will negatively impact Sertoli cell secretion of inhibin b (Damsgaard et al., 2016). Inhibin b is considered a marker for Sertoli cell function and seminiferous tubule integrity (Blevrakis et al., 2016; Damsgaard et al., 2016; Di Biscceglie et al., 2007; Plymate et al., 1992; Romeo et al., 2007). It plays a key role in the hypothalamic–pituitary–gonadal axis, reducing FSH secretion via negative feedback on the pituitary gland. Thus, by decreasing Sertoli cell secretion of inhibin b through oxidative stress negatively affecting Sertoli cells, the negative feedback mechanism is effected resulting in increased serum FSH levels (Trigo et al., 2004). This explanation supports the highly significant positive correlation exhibited between seminal ORP levels and serum FSH in this study. The following studies further support this explanation. Inhibin b positively correlated with semen parameters and testes volume, while was negatively correlating with FSH amongst fertile and infertile males (Appasamy et al., 2007). Obese males, which are expected to have seminal oxidative stress, have higher FSH/inhibin ratios compared to normal controls (Stewart et al., 2009). In addition, compared to normal controls, varicocele patients have smaller testes, lower inhibin and higher FSH levels (Pasqualotto et al., 2005). Furthermore, the amount of change in hormonal level and testicular volume varies according to varicocele severity. Patients with higher grades of varicocele have higher FSH, lower inhibin b and smaller testicular volume (Damsgaard et al., 2016).

Further investigations on patients after varicocelectomy suggest that inhibin b can serve as a predictor of post-surgical spermatogenesis improvement (Dadfar, Ahangarpour, Habiby, & Khazaely, 2010). Along with improved sperm parameters, inhibin b levels and testicular volume increased in patients after varicocelectomy. An increase in inhibin b was also detected in...
patients after varicocele sclerotherapy (Di Bisceglie et al., 2007). However, a study conducted by Cavarzere et al. did not exhibit any changes in inhibin b levels after varicocelectomy (Cavarzere et al., 2011).

The results of this study also included a weak positive correlation between ORP and LH without any significant change in testosterone levels. The exact mechanism behind this correlation is not fully understood, but might be due to a negative effect of oxidative stress on Leydig cells similar to that seen for Sertoli cells. It might also reflect a lower sensitivity of Leydig cells to the oxidative stress. Any changes in Sertoli cell function and inhibin b secretion should not impact LH secretion (Appasamy et al., 2007). On the other hand, different studies have yielded different results regarding the impact of oxidative stress on LH secretion. Some studies identified higher LH levels amongst males with varicocele compared to normal controls (Damsgaard et al., 2016; Trigo et al., 2004). Damsgaard et al. also showed that the grade of varicocele weakly and negatively correlates with LH and testosterone levels, suggesting that oxidative stress might negatively impact Leydig cell function (Damsgaard et al., 2016). Yet, other studies have not identified any correlation between varicocele and LH (Blevrakis et al., 2016; Pasqualotto et al., 2005; Plymate et al., 1992). Thus, further investigations are warranted to fully understand the mechanism behind the correlation between oxidative stress and LH.

The main limitation of this study is the lack of inhibin b assay. Inhibin b is not part of the routine male infertility work up in our facility, and as the study is retrospective, inhibin b could not be ordered for the patients. Other limitations include the lack of information on varicocele grade. It will be interesting to study if patients with grade 2 and 3 clinical varicocele have smaller testis and higher inhibin b. It will be interesting to study if patients with grade 2 and 3 clinical varicocele have smaller testis and higher inhibin b secretion, which will consequently lead to increased serum FSH levels. Thus, this study supports the diagnostic role of ORP in assessing testicular integrity and function.

5 | CONCLUSION

In summary, this study confirmed the negative relationship of ORP with important semen parameters. On the other hand, a negative relationship of ORP with the testicular volume and positive correlations with serum FSH and LH levels were found. These results highlight a potential causative role of oxidative stress reflected by ORP on the Sertoli cells as major cellular testicular component and hormone producer. Affecting Sertoli cells by oxidative stress might not only reduce their numbers leading to smaller testes, but could also lead to a decline in inhibin b secretion, which will consequently lead to increased serum FSH levels. Thus, this study supports the diagnostic role of ORP in assessing testicular integrity and function.

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