Understanding the physiologic role of oxidation-reduction equilibrium in semen

Oxidative stress, defined as an unbalanced state in a biologic system between oxidative and antioxidant molecules toward the oxidative end, is recognized as one of the most important mechanisms of male-factor infertility, with a prevalence of 40% in men diagnosed with infertility. Agarwal et al., in their recent article published in this issue of *Fertility and Sterility*, have suggested that measuring the oxidative reduction potential (ORP), which in essence measures the resulting potential between all of the oxidative molecules and all of the reductive molecules in any given fluid or system, would allow for inference of oxidative stress in semen and in seminal plasma of men for routine use within a clinical setting (1). The authors utilized a novel device ("MiOXSYS") for measurement of static ORP (sORP; the oxidation/reduction potential per se) as well as the antioxidant capacity reserve after complete exhaustion of all antioxidant species (cORP). Patients were grouped: 1) as healthy volunteers or infertile patients; and 2) as good ($\geq$40% motile sperm) or poor (<40% motile sperm) motility; measurements were carried out immediately on liquefaction (time 0) or after 2 hours (time 120) on whole semen and in seminal plasma. Only sORP in seminal plasma at time 120 was able to differentiate healthy control subjects from infertile men (these data are presented as Supplemental material to the article). Arguably, cORP in seminal plasma at time 0 and sORP in semen at time 120 warrant further exploration, because *P* values were not far enough from significance for an effect to be completely discarded. In terms of effects on motility, results showed that cORP levels were higher at time 0 in patients with poor motility, in both semen and seminal plasma, and that sORP values were higher at time 120 in patients with poor motility, in both semen and seminal plasma. The authors reported a receiver operating characteristic ROC curve establishing a cutoff value for good or poor motility, for both semen and seminal plasma. The promise of a quick method for assessing semen oxidative stress is quite interesting because, as the authors point out, current tests are time consuming and time sensitive, and may use up a large volume of sample.

Owing to the difficulty in determining end points in male infertility studies, surrogates of male fertility have been proposed, such as assessing sperm functional status (sperm DNA fragmentation, mitochondrial activity, acrosome integrity, ability to penetrate murine zona-free oocytes, and semen oxidative stress, to name a few). These functional tests have increased our knowledge of the biology underlying sperm function and male fertility, and they are likely better end points for male infertility studies than conventional semen analysis, because they offer better predictive values in in vivo and in vitro fertility outcome studies (2). Therefore, it is timely to introduce a test that offers a functional aspect to semen analysis and that can be applied to a clinical setting. It is noteworthy, however, that this study (and others cited by the authors), have not yet determined what level of ORP would lead to oxidative stress itself. We can not yet know, then, if the higher sORP values in infertile patients at 120 minutes are indeed sufficient to lead to oxidative stress or if they merely indicate a normal variation in semen redox balance. Moreover, the lack of a significant difference between sORP and cORP values between fertile and infertile men at time 0 seems to stem from a very high variance in the infertile group (1). The fact that male infertility derives from a number of different factors, and that oxidative stress is only one of those factors (albeit an important one) would account for this high data variability, but it limits the usefulness of ORP in separating "fertile" from "infertile" men.

Furthermore, it is not surprising that the observed power of sORP values in this study was low in discerning normal ($\geq$40%) from low (<40%) motility, with a reported sensitivity of 60% (at a 75% specificity and an observed positive predictive value of only 45%) at best (in semen): Reactive oxygen species play a major role in energy generation (glycolysis itself being initiated by oxidation of glucose molecules), which is a fundamental part of the maintenance of normal sperm motility. It has also been demonstrated in classic studies that oxidation of seminal proteins (then termed antagglutinin) inhibits their antiagglutination properties, which in turn coincides with motility onset on ejaculation (3). Even more toward demonstrating the importance of oxidative molecules in semen, the major alterations that sperm undergo after ejaculation to acquire the capability to penetrate the oocyte, such as hypermotility and acrosome reaction, to name a few, denominated in bulk sperm capacitation, are all dependent, initiated, or mediated by oxidative events (4). Therefore, quenching of oxidative activity in semen would putatively lead to reductive stress (such as what was initially demonstrated when dysregulated glutathione metabolism, and consequent reductive stress, led to protein aggregation cardiomyopathy [5]), in which sperm would ultimately not be able to achieve fertilization potential.

It is understood, then, that oxidative events play a major role in normal sperm physiology (3, 4), which leads to the inference that oxidative species will fluctuate within a normal range without phenotypic alterations to the semen sample being observed. This follows closely a classic nutrient curve, in which at low concentrations (i.e., "reductive stress") there is a loss of function, at a normal range (which is to be determined) the expected effects are observed, and at high concentrations (i.e., oxidative stress) there is a loss of function. It may be, therefore, that a conventional approach at constructing linear models will not fit our biologic data when we consider assessing sperm oxidative-reductive physiology, but rather a threshold model is needed, in which at each different threshold (i.e., between reductive stress and homeostasis, or between homeostasis and oxidative stress) a linear model could be fit.

Given, however, the multitude of factors that influence the seminal sample, ORP measures alone, which 1) do not account for stress itself (ORP only measures the redox potential) and 2) assess only one aspect of an effect that is harmful only in certain conditions, will likely fail to accurately diagnose a sample as "good" or "poor." That is why, as the authors demonstrate quite well in their Figures 2C and 2D, a cutoff value would misdiagnose a sample one-half of the time (1). As an added measure to the tests currently performed, however, it does bring the opportunity to asses a functional aspect of the ejaculate that a conventional semen analysis does not.

The article by Agarwal et al. brings an interesting opportunity to male infertility research, because it demonstrates that assessing the equilibrium between oxidative and reductive activity in a semen sample relates to some aspects of sperm quality (1). If this relates further to sperm functional quality, it may then be the case that there is a true effect of the seminal oxidation/reduction balance on specific aspects of sperm function, such as DNA integrity, mitochondrial activity, and acrosome integrity, as well as on the ability of sperm to achieve fertilization. Perhaps shedding light on the seminal redox potential itself in a routine setting will allow for better inference of fertility status, and this remains to be demonstrated. On the other hand, the possibility of studying how the redox balance affects aspects of sperm physiopathologic mechanisms is an interesting opportunity brought forth by this article.

Ricardo Pimenta Bertolla, Ph.D.
Human Reproduction Section, Division of Urology, Department of Surgery, Universidade Federal de Sao Paulo; and Hospital Sao Paulo, Sao Paulo, Brazil

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