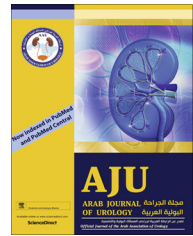




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ORIGINAL ARTICLE

Oxidation–reduction potential and sperm DNA fragmentation, and their associations with sperm morphological anomalies amongst fertile and infertile men

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KEYWORDS

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Abstract Objective: To assess seminal oxidation–reduction potential (ORP) and sperm DNA fragmentation (SDF) in male infertility and their relationships with sperm morphology in fertile and infertile men.

Patients and methods: Prospective case-control study comparing the findings of infertile men ($n = 1168$) to those of men with confirmed fertility ($n = 100$) regarding demographics and semen characteristics (conventional and advanced semen tests). Spearman rank correlation assessed the correlation between ORP, SDF, and different morphological indices. Means of ORP and SDF were assessed in variable levels of normal sperm morphology amongst all participants.

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ABBREVIATIONS

ART, assisted reproductive techniques;
 AUC, area under the curve;
 ICSI, intracytoplasmic sperm injection;
 IUI, intrauterine insemination;
 IVF, *in vitro* fertilisation;
 NPV, negative predictive value;
 ORP, oxidation–reduction potential;
 OS, oxidative stress;
 PPV, positive predictive value;
 ROC, receiver operating characteristic;
 ROS, reactive oxygen species;
 SCD, sperm chromatin dispersion

Results: Infertile patients had a significantly lower mean sperm count (32.7 vs 58.7×10^6 sperm/mL), total motility (50.1% vs 60.4%), and normal morphology (5.7% vs 9.9%). Conversely, infertile patients had significantly higher mean head defects (54% vs 48%), and higher ORP and SDF values than fertile controls. ORP and SDF showed significant positive correlations and significant negative correlations with sperm head defects and normal morphology in infertile patients, respectively. ORP and SDF were significantly inversely associated with the level of normal sperm morphology. Using receiver operating characteristic curve analysis, ORP and SDF threshold values of 1.73 mV/ 10^6 sperm/mL and 25.5% , respectively, were associated with 76% and 56% sensitivity and 72% and 72.2% specificity, respectively, in differentiating $<4\%$ from $\geq 4\%$ normal morphology.

Conclusion: A direct inverse relationship exists between seminal ORP and SDF with various levels of normal sperm morphology. Using ORP and SDF measures in conjunction with standard semen morphology analysis could validate the result of the fertility status of patients.

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Introduction

Infertility is the inability to conceive after at least 1 year of regular unprotected intercourse. About 15% of couples report this medical problem [1]. Globally, 30–50% of infertility cases are due to a male factor, reaching up to 60% where men are directly or partially responsible for the infertility [2]. Clinicians rely on conventional semen parameters for the initial evaluation of male fertility potential, and sperm morphology assessment is one of its cornerstones. Several approaches and classifications for sperm morphology assessment have been used including the strict criteria of the WHO classification, and the modified David classification [3–5]. The most recent fifth edition of the WHO manual recommends using strict criteria for examining sperm morphology, and provides a precise definition of a normal spermatozoon [3]. Multiple abnormal sperm morphology indices have been described in literature to ease interpretation of this semen parameter, including sperm deformity index (SDI), teratozoospermia index (TZI), and multiple abnormalities index (MAI). However, data on their clinical relevance remain scarce [6,7].

Sperm morphology has been subject to considerable debate about its ability to accurately predict *in vivo* and *in vitro* conception. With the exception of some specific sperm morphology defects, which are often linked to genetic disorders (e.g. globozoospermia, macrocephaly, decapitated sperm syndrome, and fibrous sheath dysplasia), sperm morphology assessment has been suggested to have very poor sensitivity and specificity in

the diagnosis of infertility [7]. Earlier studies have shown sperm morphology to be one of the most important semen parameters capable of predicting natural conception, intrauterine insemination (IUI), *in vitro* fertilisation (IVF), and intracytoplasmic sperm injection (ICSI) outcomes [8,9]. Nonetheless, more recent studies failed to confirm such propositions [10,11], where for instance, men with $<1\%$ normal forms were able to conceive without IVF, and even men with 0% normal forms had similar successful pregnancy outcome [12]. Furthermore, Hotaling et al. [11] investigated the impact of teratozoospermia ($<5\%$ normal forms) on assisted reproductive outcomes to report that isolated teratozoospermia was not associated with decreased clinical pregnancy rates in cases of IVF with or without ICSI. Hence, alternative methods such as natural conception or even IUI seem possible prior to seeking immediate IVF treatment in men with severe teratozoospermia. Such controversy highlights the uncertainty of conventional semen parameters in predicting true male fertility potential, and triggers the search for advanced tests of sperm function that could help improve diagnostic accuracy.

Seminal oxidative stress (OS) and sperm DNA fragmentation (SDF) are two advanced sperm function tests that are increasingly used in the evaluation of infertile men. OS has recently been identified as a major mediator in the various causes of male infertility [13]. High levels of reactive oxygen species (ROS) are found in the semen samples of 25–40% of infertile men [14]. Although small physiological levels of ROS are essential for normal

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