Commentary

The role of sperm DNA testing on male infertility

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Male factor is responsible for nearly 50% of all causes of infertility. Approximately 30–40% of the cases are deemed idiopathic, as there are no identifiable factors to explain the abnormal semen analysis results. Semen analysis (sperm concentration, motility and morphology) is still used for routine male infertility assessment; however, these parameters have shown to be limited as surrogate markers of male fertility. In fact, nearly 15% of patients who are suffering from male infertility have a normal semen analysis.

Impaired sperm DNA integrity affects the sperm biological structure which may ultimately result in poor pregnancy outcomes [miscarriage, recurrent in vitro fertilization (IVF) failure] in couples with otherwise unexplained subfertility. However, the sperm biological structure cannot be determined with routine semen analysis. For this, specialized sperm function tests, including sperm DNA fragmentation (SDF) and reactive oxygen species, have been utilized (1).

Sperm DNA damage may originate from the testis and/or during transit through the reproductive duct system (epididymis, etc.). The spermatozoon acquires progressive motility and fertilizing capability during its journey through the epididymis. The normal secretion and absorption function of the epididymis epithelium provides the appropriate microenvironment for proper sperm maturation. However, oxidative stress may affect the sperm chromatin during transit through the epididymis. The causative factors of SDF include paternal age, smoking, radiation, varicocele, obesity, cancers and leukocytospermia (2).

The sperm DNA integrity is essential for normal embryogenesis. Over the past decade, many articles have shown clearly that high-level sperm DNA damage is associated with poor outcomes with regard to natural conception. Moreover, SDF has been shown to be significantly higher in male patients with infertility compared to fertile counterparts. Several tests have been developed to measure SDF rates. TUNEL, SCSA, Comet assay and SCD tests are more commonly used than acridine orange (AO), aniline blue (AB), chromomycin A3 (CMA3) and toluidine blue (TB). While TUNEL and Comet provide a direct measure of breaks in the DNA, SCSA and SCD measure both the existing breaks and the susceptibility of sperm DNA to denaturation. Each of these tests assesses different aspects of sperm DNA damage, therefore, results of these tests cannot be compared with each other. Furthermore, apart from the lack of standardization it is unknown what the threshold values of these tests are.

Additionally, many other questions remain to be elucidated. For instance, what is the inter-laboratory variation? And which is the "gold standard method" (3)... Notwithstanding, sperm DNA damage has been a hot topic in the literature recently. A search in PubMed between years 2000 and 2015 showed 1,460 publications in contrast to only 100 publications between 1985 and 2000. Despite the number of publications, there is no consensus of whether or not measurement of SDF provides any clinical benefit in the assessment of the male infertility patient.

Furthermore, The Practice Committee of the American Society for Reproductive Medicine (ASRM) has not recommended the routine use of SDF tests in the assessment male infertility. On the contrary, a few studies have indicated that the evaluation of sperm DNA damage may be helpful for specific case scenarios (4). Varicocele is the most common cause of infertility and it is prevalent up to

35–40% of men presenting for male infertility assessment. Nowadays, the physiopathology of varicocele and its effect on the male infertility have not been completely understood despite the fact that several hypotheses have been asserted. Testicular hyperthermia and oxidative stress are the most accepted ones. Oxidative stress leads to both membrane function and sperm DNA damage through the generation of excessive reactive oxygen species. This phenomenon may help to understand the cause of infertility in patients with varicocele who have normal semen parameters (5).

A few treatment strategies might improve sperm DNA damage, such as intake of oral antioxidants and varicocelectomy. Several studies have demonstrated that patients with varicocele tend to have high levels of SDF than counterparts without varicocele (6). Recently, a meta-analysis involving 240 patients and 176 normal controls showed that patients with varicocele have significantly higher sperm DNA damage compared to normal control men and that varicocelectomy improved sperm DNA integrity (6).

In the paper of Agarwal *et al*., the role of sperm DNA testing in male infertility is presented using clinical scenarios. Despite the lack of strong evidence for the routine using of these tests, the authors have indicated that the measurements of SDF may be useful in some cases. They have recommended that SDF test be considered before varicocele repair in patients with high-grade (grade 2–3) varicocele who have normal semen parameters and also in those with low-grade (grade 1) varicocele with borderline/abnormal semen parameters (Grade C recommendation) (7). In contrast, both the European Association of Urology (EAU) and the ASRM guidelines have indicated that varicocele repair is not recommended in infertile patients with normal semen parameters (1). Indeed, Agarwal *et al*. cited the studies of Smit *et al*. and Ni *et al*., both of which demonstrated that patients with abnormal semen analysis and palpable varicocele had better chance for pregnancy, improvements in semen parameters and decreased SDF after varicocelectomy (8).

Another controversial indication of varicocelectomy relates to patients assisted reproductive technology (ART). Recently, Esteves *et al*. published a systematic review and meta-analysis aiming to determine the role of varicocelectomy on outcomes of ART in non-azoospermic infertile men with clinical varicocele. The mentioned study pooled 4 retrospective studies accounting for 870 intracytoplasmic sperm injection (ICSI) cycles (438 with varicocelectomy, 432 without varicocelectomy). In four studies, patient with varicocelectomy had higher clinical pregnancy and live birth rates with ICSI than untreated patients. The result of this meta-analysis suggested that varicocele repair improves ART outcomes (9).

According to the current evidence, SDF tests might be useful for infertile patients with low-grade varicocele who has borderline/abnormal semen parameters and oligozoospermic patients with clinical varicocele who are candidates for ART. The finding of high SDF levels may be helpful for deciding in favor of surgical repair. Also, SDF testing results may be an important prognostic factor for the outcomes of surgical repair. Moreover, measurement of SDF can be used for post-operative follow-up in association with semen analysis.

Another clinical scenario for SDF testing in the article of Agarwal *et al*. refers to oligozoospermic patients with repeated ART. In such cases, SDF results may be used to aid selecting the proper ART method (Grade C recommendation).

Despite the number of studies examining the role of SDF in ART, there is no consensus on its clinical utility. In accordance with the ASRM guideline, the current literature does not support a consistent relationship between sperm DNA damage and reproductive outcomes (4). However, a recent systematic review and meta-analysis suggested that sperm DNA damage is indeed related to poor ART outcomes. In the study mentioned above, Simon *et al*. have examined 41 articles, of which 16 pertained to IVF, 24 to ICSI, and 16 mixed (IVF + ICSI). Various SDF tests were used, including SCSA (23 studies), TUNEL (18 studies), Comet (7 studies), and SCD (8 studies). The results of this study showed that high sperm DNA damage was associated with reduced clinical pregnancy rates after IVF and/or ICSI. (OR 1.68, 95% CI 1.49–1.89, P<0.0001) (10).

Another recent review and meta-analysis assessed the effect of sperm DNA damage on live birth rate after ART. In this report, Osman *et al*. evaluated 6 prospective articles including IVF (1 study), ICSI (2 studies), and both IVF and ICSI (3 studies). SDF was measured by SCSA (3 studies), TUNEL (2 studies), and Comet (1 study). The authors showed that, overall, patients with low sperm DNA damage had significant higher live birth rates than patients with high sperm DNA damage. While success of IVF decreased with high sperm DNA damage, ICSI outcomes were not affected. The authors of the study mentioned above recommended ICSI instead of IVF to patients with high sperm DNA damage (11).

A number of studies have clearly shown that sperm DNA

damage in ejaculated sperm is higher than testicular sperm, thus suggesting that SDF may originate from post-testicular environment. In an animal study involving infertile mice, Suganuma *et al*., found that fertilization rates were higher when sperm obtained from testicle or caput epididymis, and that the level of sperm DNA damage was lower in sperm obtained from the testis than ejaculate or cauda epididymis. The authors' results suggest that sperm DNA damage originated during epididymal transit caused (12). Recently a prospective study aimed to investigate the effect of testicular or ejaculate sperm on ICSI outcomes in infertile men with oligozoospermia and high sperm DNA damage. The study showed that sperm DNA damage in testicular sperm was lower than ejaculated sperm (8.3% *vs.* 40.7%) and that the clinical pregnancy rate (51.9% *vs.* 40.2%), miscarriage rate (10% *vs.* 34.3%), and live birth rate (46.9% *vs.* 26.4%) favored the use of testicular sperm in preference over ejaculates sperm for ICSI (13). Another similar study investigated the effect of testicular sperm on ICSI outcomes in oligozoospermic men with who had previous ART failure with ejaculate sperm using TUNEL positive. The authors showed that the level of TUNEL positive sperm in ejaculated specimens was higher than testicular sperm (24.5% *vs.* 4.6%). Fifty percent of these patients achieved clinical pregnancy in the first ART cycle with testicular sperm (14). Results of these studies suggest that testicular sperm should be used for ICSI in infertile men with severe oligozoospermia and high SDF. SDF tests can be therefore used to choose the ART method, intrauterine insemination (IUI), IVF and ICSI, and the source of sperm for ICSI.

The final scenario of the article of Agarwal *et al*. evaluated the relationship between lifestyle and SDF in infertile men. The authors have suggested that oligozoospermic infertile patients who are smokers, obesity, and/or exposed to environmental toxicants should be assessed with SDF tests (Grade C Recommendation). The prevalence of overweight/obesity has gradually increased since 1980 and it now affects nearly 65% of the world's population. As a matter of fact, the impact of obesity on male infertility has been widely investigated. It seems that paternal obesity may lead to infertility by a multitude of mechanisms, including elevated SDF, obesity-associated hypogonadism, impaired sperm production, abnormal ejaculation, and erectile dysfunction. Recently, Campbell *et al*. published a systematic review and meta-analysis examining the effect of obesity on male infertility. The authors found that live birth rate and pregnancy viability of couples subjected to ART were lower had the father been obese. Moreover, SDF was higher in obese than in men with normal weight. However, no statistically significant difference was found between the semen parameters of obese and non-obese men (15). In contrast, Bandel *et al*., investigating the relationship between sperm DNA damage and body mass index (BMI) in 1,503 men from the general population, found that SDF levels were not associated with BMI (16). As a result, there is no clear evidence that obesity lead to infertility via high SDF.

All in all, despite the lack of strong evidence for the role of sperm DNA damage on male infertility assessment, the SDF tests might be necessary for a few specific populations. However, there is a clear need for newer studies to further address these issues.

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Footnote

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