Commentary

Sperm DNA damage diagnostics: when and why

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Sperm DNA damage is the major cause of defective sperm function. DNA damage includes DNA denaturation and sperm DNA fragmentation (SDF) and maybe the common underlying aetiology of infertility, recurrent spontaneous abortion (RSA) pre- and post-implantation losses, accelerated aging, and childhood cancer (1). Oxidative DNA damage is an important factor, which affects sperm quality and increases risk of genetic and epigenetic abnormalities. Accumulation of oxidized DNA adducts like 8 hydroxy2deoxyguanosine (8OHdG) can impair function of *denovo* methylases and result in genome wide hypomethylation which compromise genetic stability (2). Thus, loss of sperm DNA integrity not only impacts reproductive and psychological health of the infertile couple but also increases childhood disease burden. Maintenance of integrity of germ cells is therefore crucial for fertility and success in assisted reproductive technologies and for the health and well being of the next generation.

SDF occurs due to various factors like persistence of nicks created during meiosis, spermiogenesis/improper compaction, altered histone protamine ratio and abortive apoptosis (3). In addition, oxidative stress due to endogenous and various exogenous factors is one of the leading causes of DNA damage and is the major cause of loss of integrity of both mitochondrial and nuclear genome (4). It is therefore important to not only quantify the levels of SDF/DNA damage but also to determine the levels of 8OHdG to better define the aetiology of DNA damage. The latter will confirm that SDF is oxidative in nature and it will be possible to reduce oxidative stress by appropriate lifestyle modifications, intake of antioxidants, or treatment of infections and inflammation.

Using various clinical scenarios Agarwal *et al.* have documented the various conditions in which SDF testing is required (5). For example, couples undergoing IVF/ICSI with recurrent failure should undergo SDF testing. Moreover, men with advanced age, increased BMI, poor social habits like smoking, or with occupational exposure to endocrine disrupting chemicals or heavy metals are also candidates for SDF testing. In addition to men with clinical grade 2/3 varicocele who are considered candidates for varicocelectomy (5), Agarwal *et al.* advocate that men with grade 1 varicocele with normal or abnormal semen parameters but high SDF should undergo varicocelectomy as post-varicocelectomy there is a significant decline in DNA damage/SDF. It is noteworthy that the majority of factors which result in oxidative stress are modifiable like poor social habits, infection, psychological stress, intake of processed nutritionally depleted foods, sedentary lifestyle, and excessive use of cell phone (6). It is therefore important to collect a detailed social, occupational, dietary and lifestyle history of the patient undergoing infertility evaluation accompanied by a thorough clinical examination to provide appropriate therapeutics and counseling.

By virtue of its high polyunsaturated fatty acid and limited cytosolic content and thus a highly deficient antioxidant capacity, and a highly truncated DNA damage detection and repair mechanism, sperm are most vulnerable to DNA damage and is dependent on the oocyte for complete removal of damaged DNA and oxidized DNA adducts. But aged oocyte with aberrant, inefficient, imperfect repair may result in persistence of DNA lesions and mutagenic DNA bases post fertilization (7). Persistence of mutagenic bases and dysregulated sperm transcripts

may result in genetic and epigenetic abnormalities in the offspring (1,7).

DNA damage, especially oxidative damage, is due to various environmental factors and preferentially target the telomeric DNA and promoters of developmentally important genes (8). Thus, OS-induced accelerated telomere shortening and aberrant methylation may dysregulate expression of various genes critical for fertilization/implantation and early embryo development prior to activation of the paternal genome (8). Majority of antioxidants improve sperm membrane potential and its permeability and fluidity and thus improve the chances of fertilization (9). However, such sperm may still harbor DNA damage. Except for a few antioxidants, the effect of most antioxidants on nuclear damage is still controversial and high doses cause premature DNA decondensation and increase the percentage of high density staining sperm (4,10,11). Whether antioxidants impact DNA damage and translate to higher full term pregnancy rate is still unclear. However, yoga-based lifestyle intervention (YBLI) aids in combating oxidative stress by decreasing levels of free radicals and inflammatory cytokines, increasing levels of antioxidants, cell cycle repair genes and anti-inflammatory cytokines (1,12,13). In a study , Dada *et al.* showed that YBLI resulted in decline in levels of oxidative stress, normalization in levels of sperm transcripts, upregulation in activity of telomerase following 3 weeks practice of yoga and significant decline in levels of DNA damage after 6 months of practice (1,13). Thus, it is postulated that YBLI reverses/slows the rate of accelerated testicular aging by decreasing oxidative stress, DNA damage and upregulating telomerase activity (1,2,4). This simple lifestyle intervention may actually reduce the number of couples who need assisted conception and may increase the natural conception rates by lowering the levels of seminal oxidative stress and DNA damage. Thus, it is warranted that the effect of YBLI may be analyzed in a larger cohort of men with unexplained infertility.

Though sperm DNA damage does not correlate with fertilization rate, it is associated with slower cleavage, poor blastocyst morphology and a tendency to develop triploid zygotes (14). Meseguer *et al.* postulated that polyspermia is favoured when there are high levels of DNA oxidation (14), which also result in impaired cleavage and embryo asymmetry.

Therefore, identifying the cause of DNA damage is important to provide appropriate management and counseling to the couple. It is especially important to quantify DNA damage from sperm after preparation (after density gradient centrifugation) as these would give the exact SDF levels in the sample being used for assisted conception (15). Also, regular monitoring is warranted when antioxidants to prevent reductive stress as it may disrupt redox sensitive reactions, capacitation, hyperactivation, signal transduction processes, and cellular homeostasis (7).

The paternal influence on the embryo manifests even before blastocyst formation and results in developmental arrest and failed pregnancy. The spermatozoon transmits at time of fertilization not only DNA but also a host of coding and noncoding RNA. Post fertilization embryo development can be severely impaired not only by sperm DNA damage but also by dysregulated levels of sperm transcripts, shorter telomeres, and altered levels of miRNA and abortive transcripts from damaged genes (16). Bisht and Dada reported normalization of levels of dysregulated transcript following YBLI (7). Thirty to eighty percent of men with unexplained infertility have raised ROS levels, which affects sperm structural and functional integrity. The important question is whether we can predict oxidative stress and oxidative DNA damage on routine semen analysis. Tremellen *et al.* documented that poor sperm motility, teratozoospermia, high number of immature germ cells, high semen viscosity and poor sperm membrane integrity are indicators of oxidative stress in the semen (17). Early diagnosis and management of oxidative stress is important, as it is a major cause of sperm DNA damage. It has been advocated that there are lower levels of DNA damage in sperm retrieved from testis, as compared to epididymis or in ejaculate (18). However, testicular sperm are epigenetically immature and have higher aneuploidy rates and thus need to be used with caution (19). Although the levels of damage in testicular sperm is 3 to 5 folds lower than ejaculated sperm, the use of such sperm may increase incidence of imprinting defects and aneuploidies (18,19).

Four assays are commonly used to quantify DNA damage (SDF). These are direct and indirect assays namely SCSA, TUNEL, SCD, and the Comet assay. Each assay quantifies a different aspect of DNA damage, as discussed by Agarwal *et al.* who highlighted the advantage and disadvantage of each assay. There are several controversies on the assay with the highest diagnostic value. So though there are several techniques available to assess SDF, there is lack of standardization of these techniques.

Simon *et al.* reported that sperm function tests should

have the highest predictive value and thus quantifying the percentage of sperm with damaged DNA or determining the mean DNA damage of all sperm may be a prognostic factor (15). Krausz and Carrell suggested that it would be more informative if a test were done after double density centrifugation in samples being processed for ART (20). However, it would be ideal to employ a technique in which the sperm viability is not compromised (e.g., magnetic activated cell sorting or select sperm with highest net negative charge) so that such sperm can then be used for ART. Simon *et al.* documented that sperm have variable levels of DNA damage ranging from 0 to 100%. Sperm DNA damage measured after sample preparation has better diagnostic and prognostic capabilities and correlates with clinical pregnancy on Comet assay. Mean olive tail movement has the highest positive predictive value to determine successful pregnancy. Combination of various Comet parameters like percent DNA damage, OTM, and the mean number of sperm with DNA damage has highest predictive value rather than a single parameter alone (15,20). Also, DNA damage assessment should be done within a month of planning a pregnancy as the damage can vary with exposure to drugs, environmental factors, fever, infection and psychological stress.

In sperm, chromosomes occupy specific territories and are highly condensed and form hairpin shaped looped structures. Chromosome centromeres form 2–3 dusters in nuclear centre known as chromocentre. Telomeric ends of chromosome form dimers and tetramers and are situated in nuclear periphery in the nucleohistone compartment of sperm genome (21). Sperm DNA damage may favour chromosomal aberrations after first metaphase following fertilization as repositioning of paternal centromeres can interfere with normal cell division and development of embryo (22). Thus DNA damage in sperm can also induce alterations in chromosome topology and may result in reproductive failure.

To sum up, SDF testing is an important component of diagnostic workup of men with unexplained infertility. As standard semen parameters are poor predictors of fertility potential and reproductive outcome the need of the day is to supplement semen analysis with tests for assessment of oxidative stress and DNA damage.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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