Commentary

Clinical usefulness of sperm DNA fragmentation testing

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The search for the best biomarker for the diagnosis of male infertility is more evident in recent years as conventional semen analysis is not always an optimal predictor of male fertility. Since the advent of the intra-cytoplasmic sperm injection (ICSI) for male factor infertility, the pace of research related to sperm functional assays has slowed down. The haploid male gamete contributes fifty percent of the genome to the zygote, hence any perturbations at the genetic or molecular level of the spermatozoon may be reflected in the fertilized zygote, preimplantation embryo and resulting pregnancy. Chromatin organization and all its associated modifications, whether it concerns the DNA itself and/or the nuclear proteins, are critical for gene expression, cell division, and differentiation. Therefore, reproductive science researchers, as well as reproductive medicine specialists, have been keen to explore the causes of poor embryo quality, early miscarriages and unexplained infertility in relation to sperm quality. The last two decades have seen myriad of developments in the research related to oxidative stress and DNA fragmentation index (DFI) of male reproductive system, especially spermatozoa.

The essential question being asked by many in the field is whether sperm DNA fragmentation analysis adds useful information which can change diagnosis, or in understanding the prognosis better. Numerous studies have shown that sperm DNA fragmentation is an important cause of male infertility (1). However, the current literature on the predictive values of sperm DNA quality evaluation and the outcomes of assisted reproductive technology is still controversial.

The extensive compaction and remodelling of the chromatin yield a highly complicated package of DNA in the sperm head (2). DNA compaction and packaging is not an error free process. Any error during this step may also contribute to sperm DNA damage. Another potential etiology for sperm DNA damage includes abortive apoptosis and the exposure of spermatozoa to imbalance in ROS-antioxidant systems in post testicular environment (3).

Even though there are many tests that have been developed and tested for the integrity of sperm DFI, currently four of them are widely used: the Comet assay, terminal deoxyuridine nick end labeling (TUNEL) assay, Sperm Chromatin Structure Assay (SCSA), and Sperm Chromatin Dispersion (SCD) assay (4). The Comet and TUNEL assays detect DNA strand breaks while SCSA and SCD measure chromatin integrity and the susceptibility of DNA to denaturation. As Agarwal *et al.* (5) nicely explain in their manuscript, we still do not understand the true nature of DFI and what it is that the each DNA test measures (5). Even though it has been widely accepted that the SDF tests prove to have less biological variability compared to conventional semen analysis results, there exists considerable inter-laboratory variability in test results. Another confusing area is the different threshold (Cut off) values for different SDF tests. Moreover the SDF tests can be affected by the clinical conditions of the patient such as

the degree of sperm nuclear decondensation and the length of abstinence (6). The authors have given four common and important clinical scenarios where DFI plays a major role either in diagnosis or prognosis.

Multiple papers have correlated varicocele repair with improvements in DNA fragmentation rates (7). The authors explain that current evidence suggests that clinicians can better select the candidates for varicocelectomy from amongst the grade 1 varicocele (with abnormal semen parameters) and grade 2/3 varicocele (with normal semen parameters). However, the recommendations constitute low (grade C) evidence, according to factors that include the study design, the consistency of the results, and the directness of the evidence in this scenario.

Since unexplained infertility account for 10–30% of couples seeking infertility evaluation, SDF seems a useful diagnostic tool based on the few studies (8,9). As the exact causes of male infertility are poorly understood, with nearly half of all cases deemed idiopathic, sperm DFI tests prove to be important in elucidating the causes of natural pregnancy loss and IUI failures (10). If causes like smoking are corrected and if DNA fragmentation remains markedly elevated despite using anti-oxidants for a few months, serious consideration should be given to proceeding directly to IVF and ICSI instead of doing repeated cycles of superovulation and IUI which are unlikely to be successful (11).

Many studies have been done on the sperm DFI on IVF and ICSI outcomes (12). The recent meta-analysis by these authors suggests that DNA integrity test before ART is not sufficient to be a predictive index for infertile men. However, another meta-analysis indicates that there is sufficient evidence in the existing literature to suggest that sperm DNA damage has a negative effect on clinical pregnancy following IVF and/or ICSI treatment (13). Meta-analysis by Robinson *et al.* (14) showed a significant increase in miscarriage in patients with high DNA damage compared with those with low DNA damage [risk ratio (RR) =2.16 (95% CI, 1.54–3.03), P<0.00001]. A subgroup analysis showed that the miscarriage association is strongest for the TUNEL assay (14).

Oocyte quality including the efficacy to repair damages plays a pivotal role in the detrimental effect of sperm DFI and reproductive outcome. Mammalian oocytes contain a fully functional DNA repair system which may take care of persisting unrepaired damage in both the maternal and the paternal genome. Evidently, fragmentation in the sperm DNA may be repaired, but only to a certain extent. The cut-off value of sperm damage beyond which the zygote would not survive may be different in different species (15). It has become a current social trend for women to delay childbearing. However, the quality of oocytes from older females is compromised and the pregnancy rate of older women is lower (16). Hence, more studies are warranted on the effect of sperm DFI and how those are repaired by oocytes from various age groups and stimulation regimens (17). Since some recent studies shed light on the negative effect of ovarian stimulation on the protein profiling and gene expression of mammalian oocytes, future ART programs might concentrate more on mild stimulation or in-vitro maturation (IVM) based IVF (18) in select cases. Moreover, life style modification not only in male but also in female partner is also warranted prior to embarking into any assisted reproductive technologies especially when sperm DFI is high.

Jin *et al.* (19) investigated the effect of sperm DNA fragmentation (SDF) on clinical outcomes of assisted reproductive technology in women with normal ovarian reserve (NOR) versus reduced ovarian reserve (ROR). They found a statistically significant decrease in implantation rate and live birth rate in the ROR group when SDF exceeded 27.3%, but the clinical pregnancy, live-birth, and implantation rates were not affected in the NOR group. The risk of early abortion increased significantly in the NOR group when the SDF exceeded 27.3%. This is a clear indication how the oocyte quality impact the reproductive outcome based on the paternal genomic instabilities.

The accumulating data suggest that it should be possible to reduce pregnancy losses if sperm for injection could be screened for DNA damage prior to IVF/ICSI. Several promising screening methods are in development which includes electrostatic/phoretic, microscopic and biochemical techniques (20). Tests for DNA damage and selection of undamaged sperm should be considered as part of the diagnostic and treatment pathways for those suffering from recurrent pregnancy loss. Further research is required into the mechanisms responsible for and preventing the DNA damage including antioxidant therapy and use of testicular spermatozoa in place of ejaculatory sperm or frequent ejaculations prior to the day of ICSI.

Conclusions

Even though the evidence on the impact of sperm DNA integrity on reproductive outcome remains somewhat controversial, it has proven to be a useful diagnostic test in male infertility evaluation. Men with high DNA fragmentation may be more incentivised to stop smoking

or accept correction of varicoceles or other factors that may negatively impact on sperm DNA quality. If a course of anti-oxidants fails to correct the high DNA fragmentation, then, IVF/ICSI may need to be embarked on expeditiously. Occasionally, it may even be needed to resort to testicular sperm aspiration (TESA) to obtain fresh sperm with lower sperm DNA fragmentation than those in the ejaculate. There are also some data to suggest that repeated ejaculations on the same day may lower the sperm DNA fragmentation. Since the reproductive process is very complex and nature of DNA damage is variable from patient to patient along with non-uniformity of DFI tests, only future studies will define the exact role of routine sperm DNA fragmentation testing for all infertile couples.

We all recognise that "ICSI the technology before science" has become widespread as a treatment option before the basic scientists could elucidate its impact on molecular/epigenetic/transgenerational level. Hence tests like sperm DFI hold value in the treatment of infertile couples where we are still groping in darkness for the right answers. Overall, the review by Agarwal *et al.* (5) is an excellent one allowing clinicians to have a better idea how to use sperm DNA fragmentation in several very common clinical scenarios.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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