Commentary

Should sperm DNA fragmentation testing be routinely used in assessing male infertility?

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Comment on: Agarwal A, Majzoub A, Esteves SC, *et al.* Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl Androl Urol 2016;5:935-50.

Submitted Mar 29, 2017. Accepted for publication Jun 03, 2017.

doi: 10.21037/tau.2017.06.11

View this article at: http://dx.doi.org/10.21037/tau.2017.06.11

Need for sperm DNA fragmentation testing

Semen analysis (SA) is the cornerstone for male infertility evaluation. Variations in sperm quantity and quality may make SA an unreliable decision-making tool in providing an insight of infertility (1). Consequently, efforts have been made to upgrade techniques of male fertility analysis. In recent times, primary investigative techniques have centered around assessing sperm capacity, sperm function, sperm morphology and sperm nucleus. Advances in the field of male infertility research have brought about strategies for assessing sperm chromatin quality and DNA fragmentation (2).

Sperm DNA is a vital component of human conception as sperm DNA damage may affect various markers of conception including embryo quality, blastocyst development, implantation, pregnancy, and miscarriage (3).

A recently published paper by Agarwal *et al.* presented guidelines of testing sperm DNA fragmentation (SDF), and discussed its usefulness as a diagnostic tool in male fertility evaluation in various clinical scenarios. Despite insufficient clinical evidence supporting the routine use of SDF in fertility evaluation, both the American Urological Association (AUA) and European Association of Urology (EAU) guidelines on male infertility acknowledge the importance of DNA fragmentation in spermatozoa (4).

On the contrary, the Practice Committee of the American Society for Reproductive Medicine (ASRM), regardless of acknowledging the relationship between sperm DNA damage and other semen parameters, states that: '*insufficient evidence exists to recommend sperm DNA integrity test as a routine test in the evaluation and treatment of the infertile couple*' (5).

Testing methods for sperm DNA fragmentation

Sperm DNA fragmentation tests assess the quality of DNA package which carries the important genetic information of the offspring. The tests are, therefore, distinct and more significant than the conventional semen parameters (6).

The commonly used tests are; the Single-cell gel electrophoresis (Comet) assay, Sperm Chromatin Structure Assay (SCSA), the terminal transferase dUTP nick end labeling (TUNEL) assay, and the Sperm Chromatin Dispersion (SCD or Halo) test. These tests measure distinct aspects of DNA damage and have different sensitivities.

As none of suggested tests could provide an accurate indication of specific DNA sequences, this fact might have prompted the ASRM not to recommend the routine use of sperm DNA integrity tests in the evaluation and treatment of infertile couple (7).

Indications and recommendations—SDF testing

The specific utility of SDF in different clinical scenarios is likely to emerge as a useful reference for assisting practicing urologists and reproductive specialists with limited expertise in genetics, in identifying settings where SDF testing will be highly applicable clinically.

Clinical varicocele

Clinical reports have stated that a definite association exists between SDF and varicocele. Considering this, several theories have been levied to prove this fact (8).

Studies assessing SDF levels in men with varicocele

have reported prominent levels of SDF; whereas the varicocelectomy decreased SDF levels, thus resulting in improved pregnancy rates, there is very little evidence available to understand the effect of low grade varicocele on SDF (9).

High SDF has been reported in clinical varicocele, particularly grades 2 and 3; improvement of SDF in all grades of varicocele has been reported after varicocelectomy (4).

Current evidence suggests that DNA fragmentation testing may allow clinicians to select varicocelectomy candidates among those men with clinical varicocele and borderline to normal semen parameters (4).

Unexplained Infertility, natural pregnancy rates and IUI

High SDF levels are seen in men with normal semen parameters, making SDF level a valuable predictor of male fertility status. Studies have demonstrated that SDF levels can be used as a prognostic tool in predicting the likelihood of natural pregnancy (9) and high SDF is additionally associated with recurrent spontaneous abortion (RSA).

There is evidence to show that there are lower pregnancy rates in IUI patients with a SDF index >30% (10).

In vitro fertilization (IVF) and ICSI failures

Evaluation of IVF studies suggests SDF modestly affect IVF pregnancy rates. High SDF is associated with greater incidence of pregnancy loss in both IVF and ICSI (11).

Usage of testicular sperm rather than ejaculated sperm decreases the likelihood of sperm DNA damage as disulphide cross-linking of its chromatin occurs in the epididymis. Considering that most DNA damage occurs during the epididymal transit of sperm. Thus, testicular sperm has lower SDF than ejaculated sperm and higher IVF/ICSI pregnancy rates can be achieved with testicular sperm (12).

Borderline abnormal (or normal) SA with risk factors

As stated previously, oxidative stress is the key pathophysiology of male infertility. Besides, several lifestyle factors exert oxidative stress induced male infertility. Alike any other cell in the body, spermatozoa produces lesser amounts of ROS amid mitochondrial energy production. Antioxidants in the mitochondria and in the seminal fluid help to counterbalance ROS levels. Nevertheless, an imbalance may occur between ROS and antioxidants levels thus triggering a state of oxidative stress, which may harm sperm DNA (13).

Increased frequency of sperm DNA defects is often linked with advancing age. Smoking has also been shown to produce detrimental effects on conventional semen parameters, sperm fertilizing capacity and risk of infertility. Smokers have prominent levels of SDF compared to non-smokers. DNA fragmentation is also evidently higher in the infertile smokers than in the infertile non-smokers (14,15). Obesity, further is associated with abnormal semen parameters (16,17). Exposure to environmental pollutants or occupational exposure to metals like lead and cadmium are associated with male infertility (18). Organochlorine pollutants such as polychlorinated biphenyls and metabolites of dichlorodiphenyltrichloroethane is associated with DNA fragmentation in spermatozoa (19). Bisphenol A (BPA) found in plastics can alter sperm DNA integrity, sperm function, fertilization, and embryonic development via regulation and/or phosphorylation of fertility-related proteins in spermatozoa (20).

There is reasonable evidence to show the deleterious effects of high SDF in men with borderline normal/abnormal semen parameters via several mechanisms as described above.

Practical relevance of SDF testing

The evidence-based approach recommended by Agarwal *et al.*, demonstrates that SDF testing provides potential value in the evaluation of male infertility. Evidence discussed in the review indicate that improved SDF levels improve pregnancy rate and outcome.

An editorial by Drobnis *et al.* discussed that the majority of studies evaluating utility of SDF testing for diagnosis of infertility may have several shortcomings, i.e., small sample size, inadequate design, inappropriate study population and non-exclusion of female infertility. These were also highlighted recently in an ESHRE position report and in an ASRM Practice Committee guideline. Thus, there is a need for standardized techniques that compare infertile couples to a population of men with proven fertility, also excluding cases with female infertility, thus confirming the utility of SDF testing. Future research which recruits men with positive and negative SDF tests, and randomly assigns them to different treatments: expectant management *vs.* IUI *vs.* IVF *vs.* IVF–ICSI are needed which will be of great clinical value in determining SDF testing as a robust tool.

Acknowledgements

None.

Footnote

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

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Cite this article as: Malhotra V. Should sperm DNA fragmentation testing be routinely used in assessing male infertility? Transl Androl Urol 2017;6(Suppl 4):S699-S701. doi: 10.21037/tau.2017.06.11

