

Is testing of sperm DNA fragmentation (SDF) ready for the basic work-up of male infertility?

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The manuscript

The authors have presented an informative manuscript on testing of sperm DNA fragmentation (SDF). They presented background material on the development of these tests, and their discussions were supported by 129 references from PubMed up to June 2016. In the section entitled "Evidence Synthesis", they reported on 8 SDF tests that have been used clinically. The final section was called "Indications for SDF Testing" and it provided ten practical examples of how these tests were utilized in different cases of male infertility.

The authors concluded that there is fair evidence to suggest that SDF testing should be used along with the semen analyses as part of the male infertility work-up. Although this approach seems progressive, there is literature that has addressed the pros and cons of SDF testing (1). Therefore, this "Commentary" will probe the subject of SDF testing, and offer some constructive suggestions regarding practical applications of this emerging science.

So, let's begin!

In the beginning

Agarwal *et al.* pointed out that the semen analysis (SA) has been the basic test for the evaluation of male infertility for many decades. However, they noted that this test may fail to provide a complete understanding of fertility potential, and there is variability associated with repeated SAs. As a result of these limitations, other tests were developed to evaluate specific sperm functions such as the acrosome reaction,

hypo-osmotic swelling and sperm morphology, etc. In recent years, these tests have been used less because Assisted Reproductive Technologies (ARTs) enable the sperm to bypass several functions associated with fertilization.

Over the last three decades, new testing was introduced to evaluate the structure of sperm chromatin. It was suspected that these tests may provide an identifiable reason for male infertility in some cases and it was anticipated that specific clinical therapies would follow. In the beginning, classic studies concerning sperm DNA were needed to show the way. For example in 1989, Ward and Coffey (2) reported that the DNA of mammalian sperm becomes highly condensed because histones are replaced by transition proteins which are subsequently replaced by protamines, which are the final tight DNA binding proteins of sperm. Furthermore, these authors noted that mammalian sperm chromatin was so highly condensed they were extremely difficult to study with microscopic techniques. Over time, a variety of buffers were introduced to de-condense the DNA by solubilizing the protamines, and several devices were developed to read the results of the DNA damage. Some of these advancements will be presented in the next section.

The big eight lab tests

In this portion of the text, Agarwal *et al.* identified different reagents that are used to initially provoke the DNA chromatin structure, and then they described the different instruments to read and count SDF for eight separate tests. The authors pointed out that these instruments may

affect the cost of the test, because some instruments were simple microscopes whereas others were either complex scopes or specific reading devices such as flow cytometry. Furthermore, some devices required highly skilled technicians to operate them (3).

Most importantly, the authors noted that 6 of 8 tests had evidence of inter-observer variability which addressed the need for standardized protocols. Those who are interested in developing SDF testing for their own labs are encouraged to read: Sperm Chromatin Structure Assay (SCSA): 30 Years of Experience with the SCSA (4). This chapter illustrates the importance of the standardization process by calculating precision, accuracy and coefficient of variation associated with the testing.

Practical clinical scenarios & SDF

This section reports on SDF testing in 12 separate clinical scenarios. These practical applications may help the readers decide whether SDF testing is important for their practices. Although the readers are encouraged to study this section in detail, this limited "Editorial" will only briefly highlight some of the clinical scenarios to help the readers assess possible benefits of SDF testing.

Clinical varicocele

The authors acknowledged that varicoceles occur in about 20% of all men, but they reported that a substantial number of men with varicoceles conceive without difficulty. In contrast, some men with varicoceles are infertile. Surgery has been the mainstay for treatment, but until recently, varicocelectomies were often challenged in the literature because there were few prospective randomized trials. In 2012, the Cochrane Collaborative changed their position on varicocele surgery when they reviewed ten prospective randomized trials that included 894 men, because these data supported utilization of varicocele surgery (5). They concluded, "Treatment of infertile men with a clinically manifest varicocele and poor semen quality may be of benefit." This report and others have led to a resurgence of interest in varicocele surgery and research related to infertile men with varicoceles.

Recently, several new ideas regarding the pathophysiology of varicoceles have been proposed. In the past, increased testicular heat from retrograde blood flow was considered to be a principal cause of the pathophysiology. In the current text, Agarwal *et al.* indicated that varicoceles may produce increased testicular heat leading to SDF which may improve

after varicocelectomy.

In addition, other recent studies have presented new ideas associated with the pathophysiology of varicoceles. These studies have proposed that varicoceles may produce responses that are similar to all other varicose veins. For example, the retrograde flow in varicose veins were reported to transmit pressure against the walls of varicose veins which led to the release of products related to oxidative stress (6). In a clinical study among adolescents with varicoceles, Romeo and Santoro (7) were the first to document increases of Reactive Oxygen Species (ROS) and Nitrous Oxide (N₂O) that were released into the spermatic veins. These concepts have led to other articles that have encouraged specific testing for Reactive Oxygen Species with simple equipment (8), because this type of testing may identify men who may benefit from treatment with anti-oxidants (9). These findings raise additional issues. Should ROS testing be considered for the infertility work-up, as well?

Unexplained infertility/recurrent pregnancy loss, intrauterine insemination (IUI) failure

The authors mentioned that the above conditions may occur despite a normal fertility evaluation. They commented that these conditions have been found in 10–30% of couples seeking evaluations. When SDF testing was used in these cases, the chances of natural pregnancy are reduced when the SDF index, measured by SCSA, was between 20–30% and it was virtually nonexistent when the SDF index is higher than 30% (10). Among these cases, abnormal SDF test results would usually lead the couple directly to IVF/ICSI which will be discussed in the next section.

The SDF effect in IVF and ICSI

During IVF, the authors stated that the oocytes are exposed to marked oxidative stress because the intact oocytes lack defense mechanisms. In contrast, during ICSI the oocyte is protected from this attack because the oocyte cytoplasm may repair the sperm damage in association with fertilization. Thus, SDF testing seems informative among couples with persistent infertility, and ICSI should be considered in these cases whenever there is evidence of increased SDF (11).

Summary

Agarwal A. *et al.* have taken the readers into the new era of

male infertility testing. They concluded that there is fair evidence indicating that SDF testing is useful for the male infertility work-up and it should be utilized along with the semen analysis. So, where do we go from here?

In the Introduction of this “Commentary” it was noted that there were pros and cons concerning sperm DNA testing (1). In addition, several published opinion papers organized by leading medical associations and prominent investigators demonstrated varying enthusiasm for SDF testing, and they concluded that SDF testing does not reliably predict treatment outcomes and cannot be recommended for routine clinical use (12,13). Other investigators offered a more middle of the road opinion (14). They stated that although the literature is conflicting, SDF testing has the potential to become an important prognostic tool for natural and IVF/ICSI conceptions (14). Still others recommend that SDF testing along with the semen analysis is ready for routine male infertility evaluations (14,15). All of these articles presented interesting information, but they all recommend that additional robust studies are needed. However, there may be other issues to consider in future studies. Should tests for Reactive Oxygen Species be included in the evaluation of male infertility? Will additional testing add significant costs to the patients? Thus, it is clear that scientific and economic issues need further study, but Agarwal *et al.* should be congratulated for their comprehensive and informative review on the subject of SDF testing.

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Footnote

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

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