

Sperm DNA fragmentation testing reveals the overall quality of a semen sample

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We read with interest the well expressed commentary by Dr. Hallak (1) and he provided us with greater insight into different sperm DNA fragmentation (SDF) tests. Dr. Hallak highlighted an interesting point that “*having a DNA fragmentation index (DFI) of <30%, does not mean that the other 70% of spermatozoa have fully normal chromatin*” and “*this value means only that, given the physical conditions imposed on the spermatozoa to induce DNA denaturation, that 30% of the spermatozoa crossed that threshold*” (1,2). We think that it is one of the central concepts in understanding the different aspects of current SDF tests.

Although not all authors agree that Sperm Chromatin Structure assay (SCSA) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) measure the susceptibility of sperm DNA (3), they are generally classified as indirect SDF tests and are the most widely utilized and published SDF assays currently. Since indirect assays rely on pre-treatment of the specimen with acid or enzyme under specific conditions, a standardized protocol and stringent control is of utmost importance in producing reliable results which are comparable among different laboratories (4,5). Their accuracy is also attributed to the incorporation of flow cytometry. The technique allows thousands of cells to be analyzed in a relatively short time compared with the use of optical/fluorescence microscopy. The evaluation of a large number of cells is essential for SDF testing. Since every single spermatozoon in an ejaculate is probably unique in its DNA integrity, sperm

quality of a sample can only be accurately reflected when as many cells as possible are being tested. Therefore, the ability to evaluate a large cell number is another means to control inter- and intra-laboratory variations in test results in addition to a standardized protocol.

This concept also explains the lack of an absolute cut-off value for SDF tests. Semen sample from healthy fertile male has a certain amount of SDF (6). “Natural” breaks occur within the testis as an integral element of sperm protamination process. The breaks that occur at this point are necessary to replace histones by protamines. The process of protamination and sperm nuclear condensation is essential to facilitate the transmission of male genome to female genital tract (7). Presence of single stranded sperm DNA breaks may be repaired by oocyte repair machinery (8) thereby preventing adverse consequences of SDF. However, not all types of sperm DNA breaks are repairable (9). It was also shown that there is a capacity of SDF repair by oocytes. Irradiation-induced DNA-damaged sperm retained ability to fertilize the oocyte in animal model. However, oocytes had the capacity to repair SDF only up to 8%, and low rate of embryonic development and high rate of early pregnancy loss were observed beyond that level (10). As a result, there is no absolutely safe SDF level in view of multiple confounding factors including oocyte quality, and type and quantity of SDF. More importantly, different cut-off values may be required for different clinical scenarios. Therefore, low SDF does not guarantee fertility while

high SDF does not exclude possibility of fertility. A certain level of SDF such as >30% DFI by SCSA method should not be treated as an absolute value as it does not mean the remaining spermatozoa in a sample are normal. Rather, a high DFI demonstrates a poor sperm quality in a sample in general. The phenomenon is illustrated by various studies on the negative impact of high SDF on natural conception, assisted reproduction outcomes, miscarriages and genetic defects to a different degree (11). High SDF probably represents the tip of an iceberg. More studies are required to reveal the underlying type and nature of sperm DNA damage, and the correlation between SDF and other factors involved in determination of pregnancy outcomes.

The heterogeneity of human sperm illustrated the pros and cons of different treatment strategies of high SDF. The role of sperm selection techniques and use of testicular sperm in intracytoplasmic sperm injection (ICSI) has been supported by emerging evidence (12,13). However, sperm selection techniques are limited by the fact that none of them completely deselect sperm with DNA damage in view of heterogeneity of DNA damage in human sperm (14). A search for a single “perfect” spermatozoon may not be practical in the presence of high SDF. Yet, none of the sperm selection methods could guarantee bypassing the potential detrimental effect of abnormal SDF on assisted reproduction outcomes. The use of testicular sperm represents an effective treatment if post-testicular event is the major source of SDF (15). In contrast, the value of the approach in bypassing a coexisting testicular event is doubtful and the higher risk of aneuploidy in testicular sperm is a concern (16). Further research in differentiating testicular and post-testicular events possibly by co-measuring reactive oxygen species may be helpful in better patient selection. This echoes Dr. Hallak’s last statement in his commentary. Alleviation of risk factors and underlying etiologies of high SDF in a targeted therapeutic approach is always preferred and more effective. Bypassing male factor with overzealous use of ICSI should not replace proper evaluation of male infertility.

There is a need for better evaluation of infertile men. SDF testing has emerged in the last decade as a novel test offering complementary information to semen analysis. The clinical application of SDF tests not only guide our treatment decision, but also improve our understanding of the complex human reproductive system. Our current knowledge of human reproduction is only the tip of an iceberg. Tremendous effort and collaboration among

fertility specialists and researchers is needed to explore the remaining iceberg which is still submerged.

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Footnote

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

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