Editorial

A single cut-off value of sperm DNA fragmentation testing does not fit all

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We are delighted to read the insightful commentary by Dr. Evenson (1), a well-known pioneer in the field of SDF testing, in response to the practice recommendations on clinical utility of sperm DNA fragmentation (SDF) testing by Agarwal *et al.* (2).

The process of protamination during spermiogenesis was discovered as early as in the 1940s and 1950s (3,4). It is not until 1970 that the association between sperm DNA damage and reduction in fertility was illustrated (5). In 1980, Dr. Evenson *et al.* developed sperm chromatin structure assay (SCSA)—a flow cytometric assay for detection of SDF (6). In this test, the semen sample is pretreated by acid and sites of DNA strand breaks are subsequently stained by acridine orange which is a nucleic acid-selective cationic fluorescent dye. The use of flow cytometry allowed evaluation of a large number of cells rapidly by measuring the metachromatic shift of fluorescence (7). Various SDF assays, including terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and sperm chromatin dispersion (SCD), were introduced after SCSA. Following the breakthrough in laboratory assessment of SDF, the etiologies of SDF and its negative impact on reproductive outcomes were studied extensively in the last two decades. The large body of research conducted by Dr. Evenson plays an important role in our understanding of SDF and male infertility.

In his commentary, Dr. Evenson shared his perspective on the pivotal role of SDF assay in clinical male fertility assessment (1). He believes that SCSA measures existing DNA strand breaks due to the high penetrating ability of acridine orange dye to the compact nuclear chromatin structure. He discussed the use of flow cytometry in increasing precision and accuracy of SDF assessment, the importance of high correlations of SDF results between laboratories and lastly the evidence supporting the association between SDF result and natural pregnancy/assisted reproduction outcomes were succinctly summarized. Of many points highlighted by Dr. Evenson, we want to expand the discussion on one topic—the threshold value for SDF assays.

The lack of a cut-off value for SDF assays is often considered a major handicap as it casts doubts about the wider clinical application of the test. Currently, there seems to be insufficient evidence to support the routine use of SDF in male factor evaluation (8). This is mainly due to heterogeneous test methodologies and lack of rigorous SDF cut-off values in the literature (8). The quest for a clear threshold of a diagnostic test for a specific clinical circumstance would be an ideal situation. However, it is important to note that this approach is not realistic for all situations. A single magic test with a clear cut-off in the context of the complex human reproductive system is probably an oversimplification. The often coexistence of both male and female factors in an infertile couple cannot be accurately assessed by a single laboratory test on either partner. Indeed, a panel of diagnostic tests is usually required clinically for comprehensive assessment of patients nowadays. The incorporation of scoring systems or nomograms into

Table 1 Cut-off values of sperm DNA fragmentation tests for pregnancy rate prediction in neat semen samples during IVF/ICSI cycles

Study	SDF assay	Cut-off SDF index (%)
Larson-Cook 2003 (12)	SCSA	<27
Gandini 2004 (13)	SCSA	<27
Payne 2005 (14)	SCSA	<27
Bungum 2007 (15)	SCSA	<30
Speyer 2010 (16)	SCSA	<19, <30*
Simon 2014 (17)	SCSA	<27
Oleszczuk 2016 (18)	SCSA	<20
Henkel 2004 (19)	TUNEL	<36.5
Frydman 2008 (20)	TUNEL	<35
Esbert 2011 (21)	TUNEL	<36
Yilmaz 2010 (22)	SCD	<30
Anifandis 2015 (23)	SCD	<35

*, <19% for ICSI and <30% for IVF cycles. IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SDF, sperm DNA fragmentation; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; SCD, sperm chromatin dispersion.

modern clinical practice to assist the correct interpretation of multiple test results reflects the deficit of using a single clinical test. Moreover, fecundity and fertility potential should be conceptualized in terms of probability rather than a bimodal parameter and it should not be defined by a simple 'yes' or 'no'. It is also important to realize the capability of SDF assays in predicting reproductive outcomes despite the inconsistent cut-off values reported by various studies (9). Therefore, the claim to deter the clinical application of SDF testing in view of the lack of a clear-cut threshold seems less convincing.

There is good quality data on the relationship between SDF and natural conception using the excellent endpoint of time-to-pregnancy. The prospective Longitudinal Investigation of Fertility and the Environment (LIFE) study (10) and the Danish First Pregnancy Planner Study (11) not only revealed the significance of male factor in human reproduction; they illustrated the concept that fecundity starts dropping with SCSA SDF index >20% and it drops significantly at SDF index >40% (11). Although SCSA SDF index of 30% is generally accepted as the threshold, it may not represent the best strategy to interpret the test result in prediction of pregnancy outcome. In fact, SCSA SDF index between 20% to 40% should be regarded as the grey zone. While SDF indexes of <20% and >40% conveys a clearer message in prediction of natural pregnancy outcomes, the situation is less clear for men with SDF index falling into the grey zone. In these cases, clinicians should assess other confounders, including female factors, which may also play a role in determining the reproductive outcomes.

In contrast to the abundance of well conducted studies to predict natural conception from the results of SDF tests, the data in the literature is confusing when it comes to assisted reproduction particularly in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The cut-off values of various SDF tests in neat semen are summarized in *Table 1*. Despite the variation in reported cut-off values, we believe that the interpretation of a test result should not be limited by a cut-off value. In fact, a cut-off has its role in scientific studies for statistical purpose. At the same time, the nature of SDF as one of the multiple continuous variables interacting with other confounding factors in clinical practice should not be overlooked. Therefore, the approach to adopt multiple cut-off values in accordance of different scenario in clinical practice may represent another sensible way to proceed as a single cut-off value of SDF testing may not fit all.

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Footnote

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

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