Commentary

Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios

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Recent studies show that spermatozoal DNA integrity is essential for normal fertilization and transmission of paternal genetic information to the offspring. Additionally, fertilization and embryo development depend on the sperm DNA structure (1,2). Generally, while fertile men have high levels of sperm with intact DNA structure associated with normal semen parameters, infertile men have decreased DNA integrity usually associated with abnormal semen parameters (3,4). However, this association is not unequivocal, as there have been several studies indicating that increased sperm DNA damage can occur in men with conventional sperm parameters within normal reference ranges (3,5,6). Increased sperm DNA damage is defined as the percentage by which the number of cells with defects in protamination of DNA structure in the evaluated sperm cells is calculated and expressed as the sperm DNA fragmentation index (DFI) (3). It is generally accepted that there is a significant negative correlation particularly between the abnormal sperm morphology percentage and DFI (3,6). In the present study, the authors evaluated the diagnostic importance and the clinical significance of sperm DFI tests in infertile males with different clinical conditions (7).

Since varicocele is one of the most common causes of male infertility, the relationship between varicocele, sperm DNA integrity and abnormal DFI was evaluated in the study. It was reported that 11.7% of infertile males with normal semen analyses were also detected to have clinical varicocele in previous studies (8,9). The main cause of male infertility due to varicocele is oxidative stress and increased DFI (10,11). As the authors of the study have noted, recent meta-analysis showed that varicocelectomy improved sperm DNA integrity, and the mean difference of sperm DFI was reported to be approximately 3.37% (7,9). However, Agarwal *et al.* recommended surgery in cases of elevated sperm DFI tests in grade 2 and grade 3 with normal sperm parameters and in grade 1 cases with borderline or abnormal seminal parameters (7). According to international guidelines, impaired seminal parameters are already indicated for operation regardless of varicocele grade (12,13). Therefore, we conclude that, in addition to the authors' suggestion, sperm DFI tests should be recommended to determine varicocelectomy in patients with normal seminal parameters regardless of varicocele grade and in cases of previous ART failure. Increased DFI is expected in advanced varicocele cases such as grade 3 with abnormal seminal parameters. However, evaluation of DFI tests in grade 1 varicocele cases with normal seminal parameters will contribute to the clinical use of these tests.

Effects on natural pregnancy and ART outcomes of DNA fragmentation are also discussed in this study. It has been reported that high sperm DFI has been associated with failure to achieve natural pregnancy, poor ART outcomes, and high risk of miscarriage (14,15). There are several studies about sperm DNA damage and its association with impaired fertilization, suboptimal embryo quality, delayed blastocyst formation, reduced pregnancy rates, and increased abortion rate, not only with natural conception but also after IUI/IVF/ICSI treatment (2,7,14,15). In a meta-analysis involving 3,106 couples, sperm DNA damage was associated with a low pregnancy rate in IVF, but no difference in ICSI (16). In the same study, a close

relationship was determined between sperm DNA damage and repeated miscarriage with IVF and ICSI. The same findings have been corroborated by a new meta-analysis (15). When IVF and ICSI were compared, it was shown that better results were obtained with ICSI in cases involving high DNA fragmentation. Agarwal *et al.* also suggest the use of sperm DFI tests in cases with recurrent IVF failure (7). The same condition is true for spontaneous pregnancy losses and unsuccessful IUI procedures. Therefore, evaluation of sperm DNA damage in these patients and switching to ICSI in the following ART cycle should be the preferred method, as suggested by the authors. In the same meta-analysis, the effects of sperm DNA damage on live birth rate (LBR) with IVF and ICSI were also examined, and a low LBR was observed in cases with high sperm DNA fragmentation as described by Agarwal *et al.* (15).

Furthermore, a DFI greater than 30% with sperm chromatin structure assay (SCSA) method was a strong predictor for decreased pregnancy rate after IUI, while a DFI greater than 27% was recognized for IVF (2). However, a significant difference in pregnancy rates between high and low DFI rates after ICSI has not been reported (14,15). Therefore, the use of DFI tests may be recommended for ICSI indications in cases scenarios involving previous unsuccessful IUI and IVF procedures. However, the use of DFI tests is still unclear in cases of ICSI. The same findings are emphasized in Agarwal's study, and although fertility and abortion rates were not different, pregnancy rates were found to be better when ICSI was performed on cases with high DFI (7). However, in a recent meta-analysis, it was reported that sperm DFI tests had little difference in predictive value for either IVF or ICSI, and provided insufficient evidence for the prediction of pregnancy in ART cases (17).

Recently, it has been reported that DNA damage begins in the seminiferous tubules after the sperm is expelled from Sertoli cells as a normal result of the apoptotic process and chromatin compaction, and progresses gradually during the movement of spermatozoa out of the testicle and through the epididymal channel (18). However, the testicular DFI ratio is 5-fold less than in the ejaculate. For that reason, Agarwal *et al.* recommendation of using testicular sperm in preference over ejaculated sperm in failed ICSI cycles involving partners with high SDF in semen, has been endorsed by others (19).

In their present study, Agarwal *et al.* recommend SDF tests to infertile couples for lifestyle modification after the initial evaluation (7). Weight loss, regular exercise, stopping smoking, changes in diet as well as antioxidant treatment can be advised to these couples.

Although there are several studies about the clinical usage and efficacy of sperm DFI tests, there are some limitations in clinical practice. The use of several tests for the investigation of sperm DNA integrity in laboratory practice is one of these limitations. The tests are direct {terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) assay and indirect tests [comet assay, SCSA and sperm chromatin dispersion (SCD)] tests} (3,7). However, none of them is accepted as a gold standard test in the ASRM Practice Committee report (20).

Additionally, in meta-analysis, different cut-off values have been reported in the methods used (2,14). It was reported that when DFI exceed 30% with the SCSA test, it indicated a probability close to zero for fertilization with natural pregnancies and IUI. This cut-off value was 27% for the SCSA test, 30% for the TUNEL assay, and 50% for the COMET assay when IVF was used. Therefore, the cut-off level for high DFI varied in different methods. Additionally, sperm storage and separation techniques also affect sperm DNA fragmentation.

Despite the rapid progress in ART, success rates of ART remain relatively low. The development of diagnostic tests to specifically identify and isolate spermatozoa with intact DNA will be an important step in improving the reproductive outcome. In addition to oral antioxidant medications, varicocele repair, recurrent ejaculations, some novel techniques mentioned below have been used in clinical practice for this purpose (3). Recently, the use of testicular sperm with TESA/TESE/Micro TESE in repeated ART failure patients and/or using high magnification (X7200) for sperm selection (IMSI techniques) in severe OAT cases/ repeated ART failure cycles has achieved a higher success rate than IUI/IVF or standard ICSI procedure (3).

In conclusion, there seems to be a consensus that increased sperm DFI is associated with a lower chance of pregnancy with natural pregnancy, IUI and IVF, but not ICSI. Despite the rapid development of artificial reproductive methods, repeated implantation failure, inadequate response to surgical treatment of varicocele, and the inadequacy of standard laboratory tests/semen analysis to demonstrate the cause of idiopathic male infertility prompted the clinical usage of advanced sperm tests. Among these tests, sperm DNA fragmentation tests have been recommended as diagnostic tests in cases of repeated implantation failure, for the decision to perform varicocele

surgery before going to ART treatment, and/or in patients having unsuccessful ART outcomes despite the sub fertile or normal semen analysis parameters, and/or prior to the decision to adopt invasive methods such as the use of testicular sperm for ICSI.

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Footnote

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

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