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Clinical uses and implications of sperm DNA fragmentation assays

Abstract

Although sperm DNA fragmentation (SDF) is correlated with infertility, recurrent pregnancy loss (RPL), and childhood morbidity, the utility of SDF assays as part of the patient's evaluation has been debated. Proponents of this measure suggest that SDF assays can guide the management of infertile couples with repeated IVF failure, couples with unexplained infertility, infertile couples with high SDF risk factors, infertile men with varicocele, and couples with RPL. On the other hand, critics question the added value of SDF indices. The cost and controversy of SDF assays have resulted in its low utilization. The aim of the current review is to determine which patient populations would most benefit from SDF assays and how SDF can guide the management of infertility.

Keywords: Sperm DNA fragmentation, IVF Failure, ICSI, recurrent pregnancy loss, varicocele, unexplained infertility

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Abbreviations: SDF, sperm DNA fragmentation; RPL, recurrent pregnancy loss; SCD, sperm chromatin dispersion; TUNEL, TdT-mediated dUTP nick-end labeling; SCSA, sperm chromatin structure assay; IVF, *in vitro* fertilization; ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection

Introduction

Approximately half of infertile couples are diagnosed with male factor infertility. In addition to the routine semen analysis, some clinicians advocate for sperm DNA fragmentation (SDF) as an advanced complementary method for certain infertile couples.[2] Since the male gametes lack many DNA repair mechanisms, sperm DNA is especially susceptible to damage by oxidative stress from neighboring immature sperm, leukocytes, and other environmental exposures.[3–5] SDF is clinically significant for its correlation with infertility and recurrent pregnancy loss (RPL).[1] However, tests that measure DNA fragmentation can be expensive, and the clinical value of its utility has been highly contested. This review aims to identify the couples who would benefit from SDF assays and the role that SDF can play in the management of infertility.

Discussion

SDF Assays

The comet assay, sperm chromatin dispersion (SCD) test, sperm chromatin structure assay (SCSA), and TdT-mediated dUTP nick-end labeling (TUNEL) assay are the most commonly employed tests for SDF. The comet assay uses a gel electrophoresis to compare the ratio of the smaller DNA fragments (tail of the comet) from the intact chromatin (head of the comet). While this test is sensitive, it is also operator-dependent.[6] The SCD uses fluoroscopy to dye intact DNA, and unlike the other assays, SCD quantifies the normal DNA rather than the DNA fragmentation. The primary advantage to SCD is that it comes in an inexpensive, accessible kit, but clinically significant correlations between this method and treatment outcomes are limited.[7]

The sperm chromatin structure assay (SCSA) indirectly measures chromatin integrity by dying single and double DNA strand breaks and quantifying the result with flow cytometry. While standardization of SCSA has made this technique highly reliable, it is cost prohibitive for many medical centers. The TUNEL assay is similar to the SCSA, but instead of dying the DNA strand breaks, fluorescently marked dUTP is enzymatically incorporated into damaged DNA. In spite of the lack of standardization of TUNEL protocol, this assay is the preferred method of testing due to its established clinical value.[7]

Clinical Guidelines

SDF analysis may guide management of several infertile populations. As only a small percentage of sperm reaches the ovum, SDF indices in the general population are not a useful measure and should also not be used in the initial infertility workup. Cho et al.[2] recently published the following list of clinical indications for when to test SDF as seen in Table 1; couples with repeated *in vitro* fertilization (IVF) failure; couples with unexplained infertility; infertile couples with risk factors, such as smoking, high fat diet, ethanol consumption, and radiation exposure; infertile men with a clinical varicocele; and couples undergoing a RPL workup.

Table 1 Clinical indications for SDF testing

Current clinical indications for SDF testing
Repeated IVF failure
Unexplained infertility
Infertility with SDF risk factors
Infertility with clinical varicocele
Recurrent pregnancy loss workup

SDF may assist in the treatment of couples with repeated failure of IVF and intracytoplasmic sperm injection (ICSI) treatments. Meta-analyses revealed SDF was negatively correlated with pregnancy rates and live birth rates in IVF and ICSI.[8,9] Moreover, SDF was also

correlated with miscarriage rates in IVF and ICSI.[10] However, the clinical use of SDF analysis in routine IVF treatment is limited as SDF does not have predictive value in pregnancy or the difference between IVF and ICSI outcomes.[11] Therefore, current guidelines suggest that sperm chromatin analysis should only be offered to couples with recurrent IVF failure and those with unexplained infertility.[2] Moreover, a potential intervention is being investigated for those who failed several cycles of IVF and have high SDF. In a study done by Datillo et al.[12] antioxidant supplementation of vitamin E and acetylcysteine was given to couples with high SDF and recurrent IVF failure. A statistically significant reduction in SDF was observed, and 20% of the couples achieved spontaneous pregnancy, and the live birth rate increased to 39.3%.[12] While SDF indices are associated with negative IVF and ICSI outcomes, antioxidants may play a role in the treatment of repeated IVF failure in couples with high SDF.

In couples with unexplained infertility, SDF may aid in determining testicular extraction candidacy. A study conducted by Esteves et al.[13] found that men with oligozoospermia and high SDF had higher live birth rates from ICSI with testicular sperm when compared to ICSI with ejaculated sperm (46.7% vs 26.4%, p=0.007). A meta-analysis also by Esteves et al.[14] determined that the rates of clinical pregnancy and live birth were higher in ICSI with testicular sperm than with ejaculated sperm in men with high SDF (OR 2.42, p<0.001 and OR 2.58, p<0.001, respectively). Therefore, SDF should be considered in couples with unexplained infertility to determine if they are good candidates for ICSI with testicular sperm.

Additionally, couples with unexplained infertility may benefit from lifestyle intervention. Clinical guidelines also suggest that couple with lifestyle risk factors should have a SDF assay to encourage on behavior modification and track SDF improvement.[12] The lifestyle factors correlated with high SDF include smoking, a high fat diet, alcohol consumption, and radiation exposure. Smoking has been linked to poor sperm quality as well as high SDF in certain populations.[15,16] Though the research is limited on the effect smoking cessation has on SDF, smoking cessation should always be encouraged. A high fat or "Western" diet is clearly linked to high SDF.[17] However, rat models have demonstrated that diet, exercise and antioxidant supplementation may reduce the damage caused by a former high fat diet.[18] Such lifestyle interventions should be encouraged in men with high SDF who consume a high fat diet. Chronic ethanol exposure has been correlated with high SDF in men[15,19] and experiments with mouse[20] and rat models[21] concurred with this finding. Couples with high SDF and infertility may benefit from alcohol abstinence. The final lifestyle factor is radiation exposure, and the most common radiation exposure is cell phone use. While it is well accepted that cell phone use increases SDF, men with existing fertility problems may be more susceptible to the damage.[22–24] Men with unexplained infertility and high SDF should be counseled to not carry their cell phone near their groin. In combination with a thorough history, SDF can guide the clinician on counseling the couple on lifestyle modifications that may increase the likelihood of a successful pregnancy.

Varicocele is a state of high testicular oxidative stress, which can lead to male factor infertility due to impaired sperm function and high SDF.[25] Varicocelectomy may improve sperm quality in some patients, and determining which patients will not have clinical benefit from the surgery will prevent an unnecessary procedure. A recent meta-analysis concluded that infertile men with a subclinical varicocele (grade 1) do not benefit from a varicocelectomy, but a varicocelectomy may improve live birth rates and reduce miscarriages in men with a clinical varicocele and abnormal sperm parameters.[26] However, this meta-analysis did not look at SDF specifically. In a small study performed by Ni K et al.[27] varicocelectomy reduced the SDF in men with astheno/oligozoospermia and a clinical varicocele but not those with normozoospermia and a clinical varicocele or in those with a subclinical varicocele. Ni K also observed that pregnancy rate was negatively correlated to SDF in the astheno/oligozoospermic group (p<0.01) and the normozoospermic group (p<0.05).[27] In summary, infertile man with varicocele and high SDF may benefit from surgical intervention.

Finally, clinical guidelines also suggest analyzing the SDF in couples with RPL. SDF is significantly correlated with couples with RPL.[28] In addition to counseled on the same lifestyle interventions as mentioned above, preliminary studies suggest that antioxidant supplementation may play a role in the treatment of RPL. In one case-control study that was too small for statistical analysis, daily administration of an antioxidant cocktail helped couple with RPL achieve a live birth at term.[29]

Conclusion

In conclusion, SDF is may be useful in guiding the management of selected populations as patients with repeated IVF failure, unexplained infertility, risk factors for high SDF, clinical varicocele, and RPL. Risk factors for high SDF include smoking, a high fat diet, chronic ethanol exposure, and radiation. Antioxidant supplementation and lifestyle modification can be considered for infertile couples with high SDF. The cost of the assays, patient anxiety, lack of clinical value in the general population, and lack of standardization of the TUNEL protocol are the main disadvantages to SDF analysis. Future work should examine the effects of antioxidant therapy and lifestyle modification on pregnancy rates in infertile men with high SDF.

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Conflict of interest

Authors declare there is no conflict of interest in publishing the article.

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