

## Dependability of the Sperm Chromatin Dispersion (SCD) Assay for Recurrent Sperm DNA Damage Evaluation of Sperm DNA Fragmentation (SDF) in Men with Presentation of Infertility-A Short Communication

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How the male attributes in the generation of a healthy embryo, there exists a need for a sperm that is of good quality with minimum genome injury whose estimation is usually done by the determination of sperm Deoxyribonucleic Acid (DNA) breakdown or the Sperm DNA Fragmentation (SDF). SDF usually guides with regards to the quality of sperm chromatin in case of men with a presentation of infertility. The causes attributed are the capacity of side actions of escalation of SDF amounts on natural fertility, embryo generation along with implantation, risk of miscarriage along with health of the newborn [1]. Earlier we detailed the role of SDF in Intracytoplasmic Sperm Injection (ICSI) with use of Time Lapse Microscopy (TLM) simultaneously [2]. SDF represents a marker of chromatin injury, takes place at the time of spermiogenesis, halt in the epididymis along with post-ejaculation in view of various factors that are not unique like varicocele, insufficient life style like toxic exposure secondary to occupational along with experimental factors, aging as well as infections [3]. For the birth of a healthy newborn, genomically healthy sperms are necessary, while enhancement of sperms with injured chromatin in the human ejaculate might result in non-stabilization of the genome at the time of fertilization as well as embryogenesis [4]. Thus it has been pointed that the evaluation of sperm chromatin at a molecular level might aid in its provision of the paternal factor for infertility, besides point to decide what treatment actions might be feasible for enhanced sperm chromatin quality [5].

A key action of the semen evaluation with regards to clinical andrology laboratory is if estimation of a variable by a single investigator in the same person at 2 separate time periods gives the akin results. Considerable interindividual differences with regards to routine semen factors has been well revealed [6]. These differences get reasoned out by environmental, technical along with biological factors [7]. Conversely, studies that evaluated the intrapersonal differences of SDF over time duration are rare [8], with no study documenting the utilization of Sperm Chromatin Dispersion (SCD) assay. This SCD is dependent on the basis that sperm along with sperm DNA fragmentation are incapable of generation of a typical halo of the dispersed DNA loops that is classically seen in nonfragmented DNA [9]. In case of SCD sperm suspension are embedded within agarose gel on slide along with treated with a solution that is acid denatured for the production of a limited single strand DNA motifs at the region of the, existent single or double strand breaks. The denaturation is halted, along with spermatozoa getting exposure to a solution

that is lysing for deletion of the sperm membrane along with nuclear proteins. Finally, the slides get stained, along with the proportion of sperms with nondispersed along with dispersed chromatin loops get manually evaluated under fluorescence or bright field microscopy [1,10]. The typical halos are akin to the open loops that are attached to the nuclear structure as visualized in sperm with low or no SDF. Conversely sperms revealing SDF display minimum or no halos.

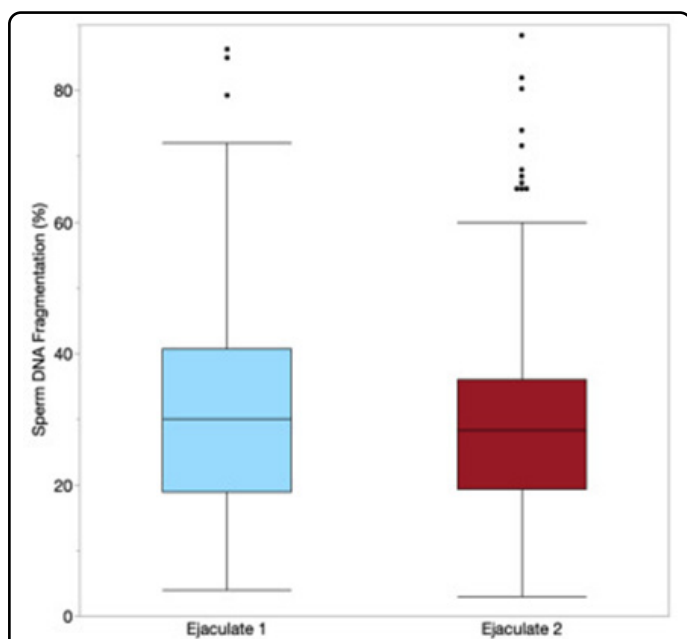
Enough intactness of DNA is key for achieving successful fertilization, generation of embryo, implantation, along with pregnancy development, in view of a minimum of 50% of the embryonic genome being accorded [10]. Thus the sperm DNA intactness is hence believed to be a significant marker of the fertility capacity of the spermatozoa. Multiple intrinsic in addition to extrinsic risk factors for SDF are existent that are inclusive of abnormalities in the chromatin condensation, apoptosis that is abortive, as well as Oxidative Stress (OS) [10]. Hence having a SDF testing that is trustworthy is necessary for the assessment with regards to the treatment of a couple whose presentation is infertility.

More recently, Esteves et al. [11], described a prospective study where assessment of how much trust could be laid on the sperm chromatin dispersion test for the assessment of sperm fragmentation. Their publication was inclusive of a concentrated patients population, the sample size that was enough, the experimental protocol that was well described in full details, negative along with positive controls besides statistical detailing along with utilization of the particular statistical tools in details. The aim of Esteves et al. [11], was the assessment of intra individual consensus of the sperm chromatin dispersion test outcomes for evaluation of SDF in men presenting with infertility.

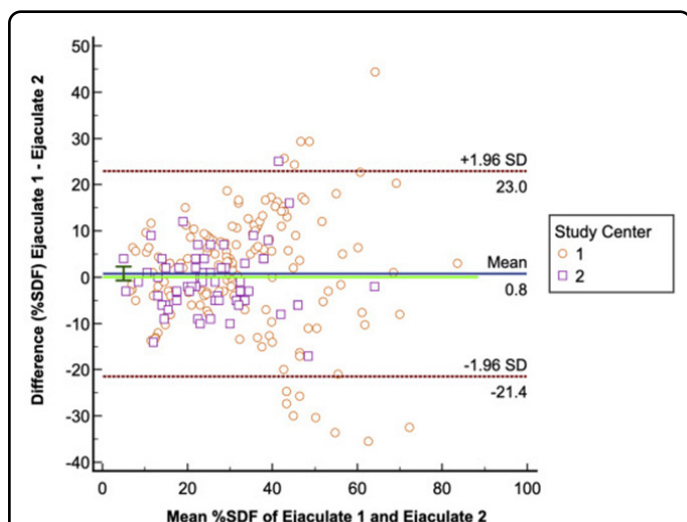
They did a classification of DNA fragmentation into 3 namely

- i. Normal (<20%)
- ii. Intermediate (20-30%)
- iii. High (>30%)

They illustrated that lower differences in recurrent investigations, in normal or high DNA fragmentation, since >80% of recurrent samples as per these classifications continued to remain in the same subgroup or category. The ones where alterations actually occurred, did so in the intermediate class. Conversely, 33% of men with infertility presentation with the prior intermediate DNA fragmentation altered to the normal of high subgroups of SDF on recurrent investigation (Figure 1 and 2).



**Figure 1:** Boxplots showing the distribution of sperm DNA fragmentation levels assessed by the sperm chromatin dispersion test according to the first and second ejaculates of 219 men with infertility. The boxplot includes the median (horizontal line in the box), 25%-75% interquartile range box (i.e., representing 50% of the data), minimum and maximum values, excluding outliers (whiskers extending outside of the box), and outliers (black dots). The median sperm DNA fragmentation values between the first and second ejaculates are not significant (Wilcoxon test,  $P=0.24$ ).



**Figure 2:** Bland-Altman diagram showing the plot of the difference between the results of the SDF testing (by sperm chromatin dispersion, %) in two separate ejaculates against the mean of the pair ( $n=219$ ). The red dotted lines show the limits of agreement. The purple line shows the mean value of the differences; it is 0.80%, indicating that on average, the % SDF from the second test was smaller than that of the first test. The green line is the zero line used to assess the discrepancy of the observed mean difference from zero. No funnel effect is observed, and the diagram shows that approximately 95% of the differences in the studied subjected lay within the limits of agreement, indicating acceptable reliability between the test pairs. SDF=Sperm DNA Fragmentation.

Hence this publication illustrated as per the statistical language a clinical observation that possesses the capacity of being significant with regards to existence of cases where the SDF value impacts the decision made by the treating medical physician. Like there is an existence of a documented part for testicular sperm extraction in a setting where earlier In Vitro Fertilization(IVF) had failed with the male possessing high SDF in his ejaculated sperm.

The existent work of Esteves et al. [11], gives us an opportunity of contrasting the SDF observations in the same person subsequent to various treatment protocols, like in testicular sperm extraction, varicocele surgery along with utilization of anti oxidants, with their confidence sustenance that variation with time duration would not bother the data. In a systematic review that got carried out by Zini as well as Dohle [12], tried quantitation of association amongst varicocele correlated SDF along with SDF control. Assessment of 7 studies was performed as well as their observation was that of 6/7 studies ( $n=279$  patients), patients that possessed a varicocele illustrated significant higher SDF in contrast to fertile controls.

Moreover, Wang et al. [13], in a follow up publication documented a meta-analysis of 6 studies ( $n=177$  patients), whose presentation was the action of varicocele correction on alterations of the concentrations of the DNA injury. As per these workers varicocele correction significantly resulted in enhancement of DNA intactness. Such outcomes can be depended upon with greater reliance with the knowledge that repeated SDF prior as well as subsequent, to any kind of treatment does not impact by recurrent investigation.

Usually men are seen following a failed IVF with female assessment having been normal along with a normal semen assessment as per the World Health Organization (WHO) criteria. Additionally, functional sperm tests like SDF can give extra knowledge along with isolation of patients for aiding in finding the etiology of infertility. With this information that there is existence of low intrapersonal difference with SDF now gives provision of trustfulness in the clinical significance along with the anticipation value of this laboratory investigation [14].

## Conclusion

With this information that there is existence of low intrapersonal difference with SDF now gives provision of trustfulness in the clinical significance along with the anticipation value of this laboratory investigation (SCD) [12,14]. Hence the SCD is a dependable test with intrapersonal difference of the persons that conducted the tests usually are similar other than occasional difference.

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