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A High Sperm DNA Fragmentation Index Negatively Affects Pregnancy Outcomes in ART

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Abstract

Background: Sperm Chromatin Structure Analysis (SCSA®) is a patented assay that has emerged as an effective method for assessing sperm DNA quality in infertile males. It is demonstrated to hold significant importance in the decision-making process for employing various assisted reproductive techniques (ART), primarily due to its capability to predict pregnancy outcomes based on the extent of sperm DNA damage.

Objective: The purpose of this study was to determine if high sperm DNA fragmentation affected pregnancy outcomes in ART cycles.

Methods: This retrospective observational study presents the most extensive study survey to date within the Indian sub-continent, involving a cohort of 574 patients reporting to a Fertility Centre between 2014 and 2018.

Results: Analysis of 574 patients undergoing ART procedures revealed no significant difference in sperm count, motility, or male/female age between those achieving positive pregnancy outcomes and those who did not, emphasizing the multifactorial nature of fertility. Increased DNA fragmentation (%DFI) correlated with reduced pregnancy success, emphasizing the importance of assessing sperm DNA integrity.

Conclusion: The broad implications of this research lie in providing fertility specialists with a reliable and dependable tool to personalize ART approaches based on sperm DNA quality, thereby optimizing the effectiveness of ART cycles.

Introduction

The influence of sperm DNA fragmentation on the outcomes of assisted reproductive treatments (ART) remains a topic of ongoing debate. The quality of the sperm and the integrity of the sperm chromatin identified as the sperm DNA fragmentation index (%DFI) is an important indicator of pregnancy outcome [1-8]. Sperm DNA fragmentation refers to the presence of single- and double-strand DNA breaks, which are commonly found in sperm and can affect natural reproduction [9-10]. These studies have proposed that tests designed to measure sperm DNA fragmentation can effectively differentiate between fertile and infertile men, although it is important to note that various methodologies and criteria are used in these studies. It is recommended that individuals experiencing recurrent pregnancy failures consider undergoing assays to detect sperm DNA damage.

The Sperm Chromatin Structure Assay (SCSA®), regarded as the gold standard for appraising sperm DNA fragmentation, generates a remarkably consistent %DFI measurement [11], and patients can be placed into distinct fertility potential categories based on their %DFI readings. The SCSA® is recognized in the scientific literature for its utility in assessing sperm DNA fragmentation, which is an important factor in male fertility. While SCSA® is highly regarded for its specificity, accuracy, and predictive value regarding fertility outcomes, we acknowledge the field of sperm DNA integrity testing includes several methods, each with its strengths and limitations. These methods include the TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labelling), Comet assay, and SCD (Sperm Chromatin Dispersion test), among others. Particularly, with the use of SCSA®, several studies have indicated the probability of a successful pregnancy outcome experiences a steep decline with % DFI surpassing 25%, particularly when female infertility factors are excluded [1, 12-14]. In such cases, clinical intervention through in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) is suggested, contrasting with situations where % DFI falls below 25%, favouring in vivo and intra-uterine insemination (IUI) [15-16].

Based on these criteria, several fertility centres in India have adopted the SCSA® testing, particularly in cases of implantation failure and repeated miscarriage (www.andrologycenter.in). We present the largest single-centre study to date that uses %DFI as a standard criterion for sperm quality analysis and the potential of utilizing SCSA® %DFI as a 'biomarker' of fertility outcomes in patients with male factor infertility and the enhanced utility of this test compared to conventional sperm parameters.

Methods

A total of 941 patients data reporting to a fertility centre (GG Hospital, Chennai) from 2014-2018 underwent a standard semen analysis following written consent with clinical outcome data collected following intra-cytoplasmic sperm injection (ICSI) cycles. Following data collection, a data clean-up was performed.

Inclusion criteria

The patients for whom final pregnancy/delivery outcomes data was available, patients who underwent the complete ICSI cycles, and patients with both %HDS and %DFI data available.

Exclusion criteria

It includes ongoing pregnancies, cancelled cycles, and outpatient data.

A total of 574 patients (negative pregnancy outcome 324; positive pregnancy outcome 250 (153 delivered and 97 miscarriages) were included in the final analysis cohort. Standard semen analysis included sperm count, motility and morphology as per standard WHO norms for manual semen analysis. For the SCSA® analysis, individual cryopreserved semen samples were thawed at 37°C and then immediately cooled on crushed ice. An aliquot of semen was diluted with TNE buffer to approximately 1–2 × 10^6 sperm/ml and mixed with an acidic solution containing HCl, NaCl, and Triton X-100. The diluted HCl was prepared from commercial 2.0 N HCl. After 30 seconds, sperm were stained with an acridine orange solution at a molar ratio ensuring AO/DNA-P >= 2 and analyzed in a FACS CALIBUR™ flow cytometer (BD Biosciences, San Jose, CA, USA). The system was calibrated, and a sample flow was stabilized within 2 minutes.

Each sample was analyzed twice to assess 5000 sperm at a rate of 100-250 events per second. If the rate exceeded 250 events/s, the sample was re-prepared. Consistency was verified by comparing the DNA Fragmentation Index (DFI) across duplicates; if variance exceeded 10%, samples were reanalyzed. Standard deviations between duplicates were calculated to ensure accuracy.

Patients signed informed consent to be included in ICSI and the SCSA® %DFI was measured 1-2 months prior to oocyte retrieval. ICSI, as per routine guidelines, was used in patients who showed various manifestations of male factor infertility (OATs, Surgically Retrieved Sperm, Retrograde Ejaculation, Asthenozoospermia, Necrozoospermia, Teratozoospermia), including high sperm DNA damage; a low number of oocytes aspirated, poor oocyte quality, advanced maternal age, tubal factors; and/or poor fertilization rate and Total Fertilization failures in previous IVF cycles. Serum beta-hCG was measured 14 days after embryo transfer; a result of more than 50 mIU/mL was considered beta-hCG-positive. These women underwent a transvaginal ultrasound scan 2 weeks later. Clinical pregnancy was determined by the presence of a gestational sac and fetal cardiac activity, which were determined at 4 weeks after embryo transfer. The implantation rate was calculated based on the number of gestational sacs per embryo transferred. A biochemical pregnancy was defined as beta-hCG-positive cases without any gestational sac at 4 weeks after embryo transfer. Ongoing pregnancy was defined as fetal development at 12 weeks of gestational age. Moreover, EPL included biochemical pregnancy, anembryonic miscarriage, and embryonic miscarriage (miscarriage subgroups); this classification was based on the consensus statement of terminology of the European Society of Human Reproduction and Embryology [17].

Statistical analysis

All statistical analyses were performed using GraphPad Prism 6.0f (La Jolla, CA). The Mann-Whitney U test was performed to assess differences in sperm parameters between groups with different pregnancy outcomes and the Kruskal-Wallis test was used to evaluate variations in sperm DNA fragmentation index (%DFI) across groups. Chi-square tests were applied to explore the association between sperm motility classifications and pregnancy outcomes. All tests were conducted with a significant level set at $p < 0.05$.

Results

Based on the criteria mentioned in the previous section, 324/574 patients were categorized as negative pregnancy outcomes, 250/574 were categorized as having positive pregnancy outcomes. Amongst the 250 positive pregnancy outcomes, 153 were live births and 97 were miscarriages. The relevance of sperm count, sperm motility, male/female age, male/female factor, %DFI, and %HDS (High DNA Stainability) to pregnancy outcomes was checked.

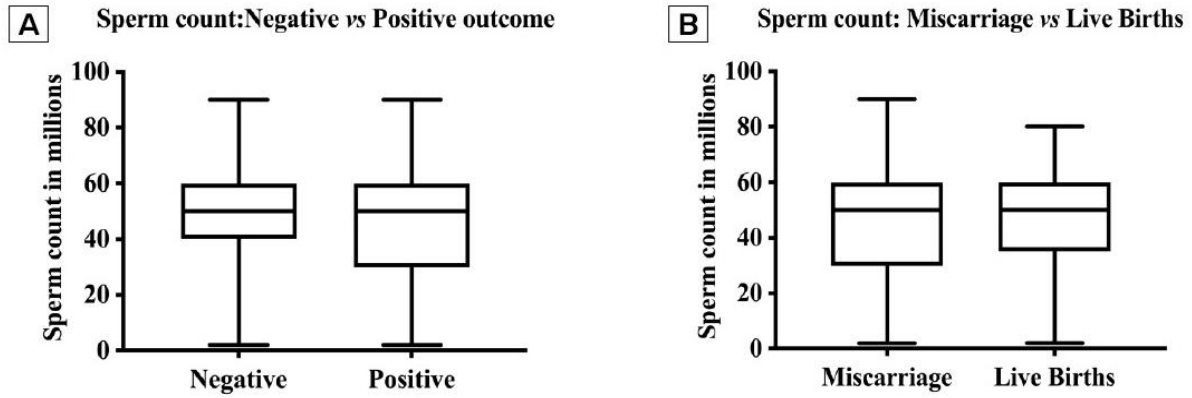


Figure 1: Sperm count A) pregnancy outcome and B) Miscarriage and Live births

A comparison of sperm count between negative and positive pregnancy outcomes [Figure 1A] was first performed followed by a comparison of only positive cases with miscarriage vs. live births [Figure 1B] to determine any differences, using the Mann-Whitney U-test. The results showed that there were no significant differences in sperm counts between these groups, as indicated by p-values of 0.523 and 0.885, respectively. Additionally, the median sperm count remained consistent at approximately 50 million across all groups.

We systematically assessed the influence of both male and female ages on pregnancy outcomes. Our analysis involved column graphs that compared the ages of both male and female patients across different groups: those with negative and positive outcomes, as well as individuals experiencing miscarriages and live births.

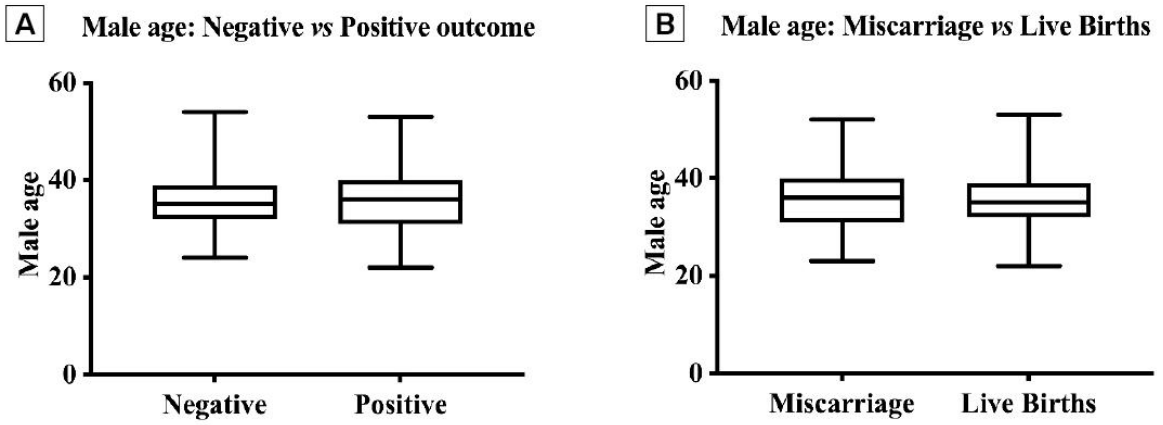


Figure 2: Male age in A) Negative vs Positive pregnancy outcomes B) Miscarriages vs Live births

Utilizing the Mann-Whitney U-test to rigorously examine these comparisons, statistical tests indicated no significant differences in either male or female age between these groups, as evidenced by the calculated p-values of 0.768 (negative vs positive) and 0.177 (miscarriages vs live births) for male age [Figure 2A and 2B, respectively], and 0.165 (negative vs positive) and 0.506 (miscarriages vs live births) for female age [Figure 3A and 3B, respectively]. Moreover, the median ages for both males and females remained remarkably consistent across all the comparison groups.

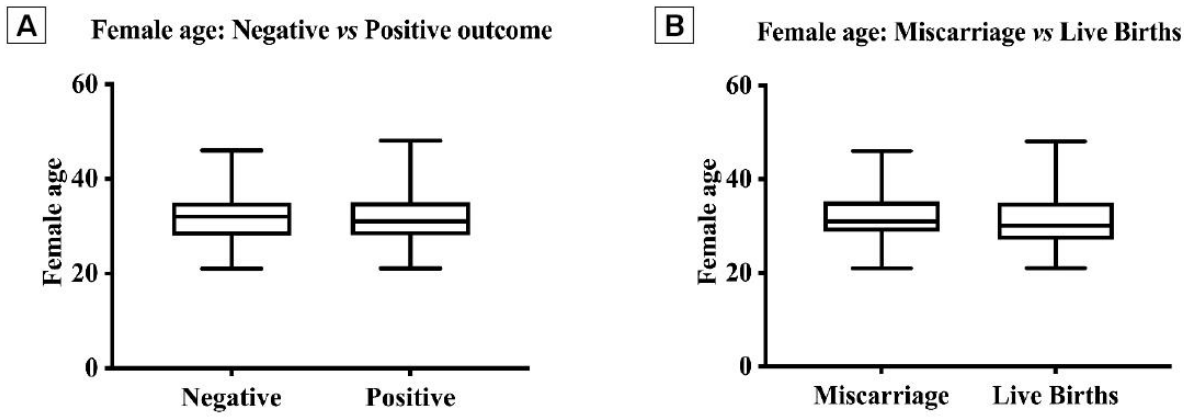


Figure 3: Female age in A) Negative vs Positive pregnancy outcomes B) Miscarriages vs Live births

We then conducted an analysis to investigate the relationship between sperm motility and pregnancy outcomes. We defined "normal motility" as having more than 40% of sperm categorized as "motile", while conversely, having less

than 40% of sperm classified as non-motile was considered indicative of asthenozoospermia, a condition characterized by reduced sperm motility, as per the standard WHO 5th edition guidelines.

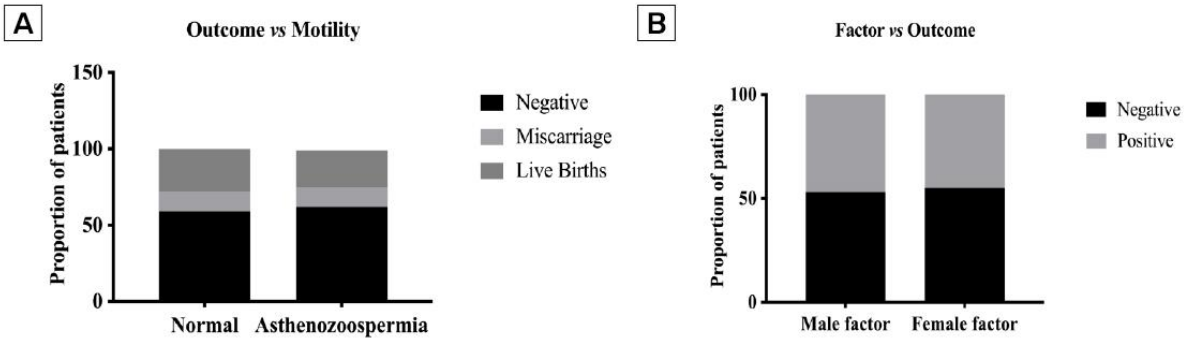
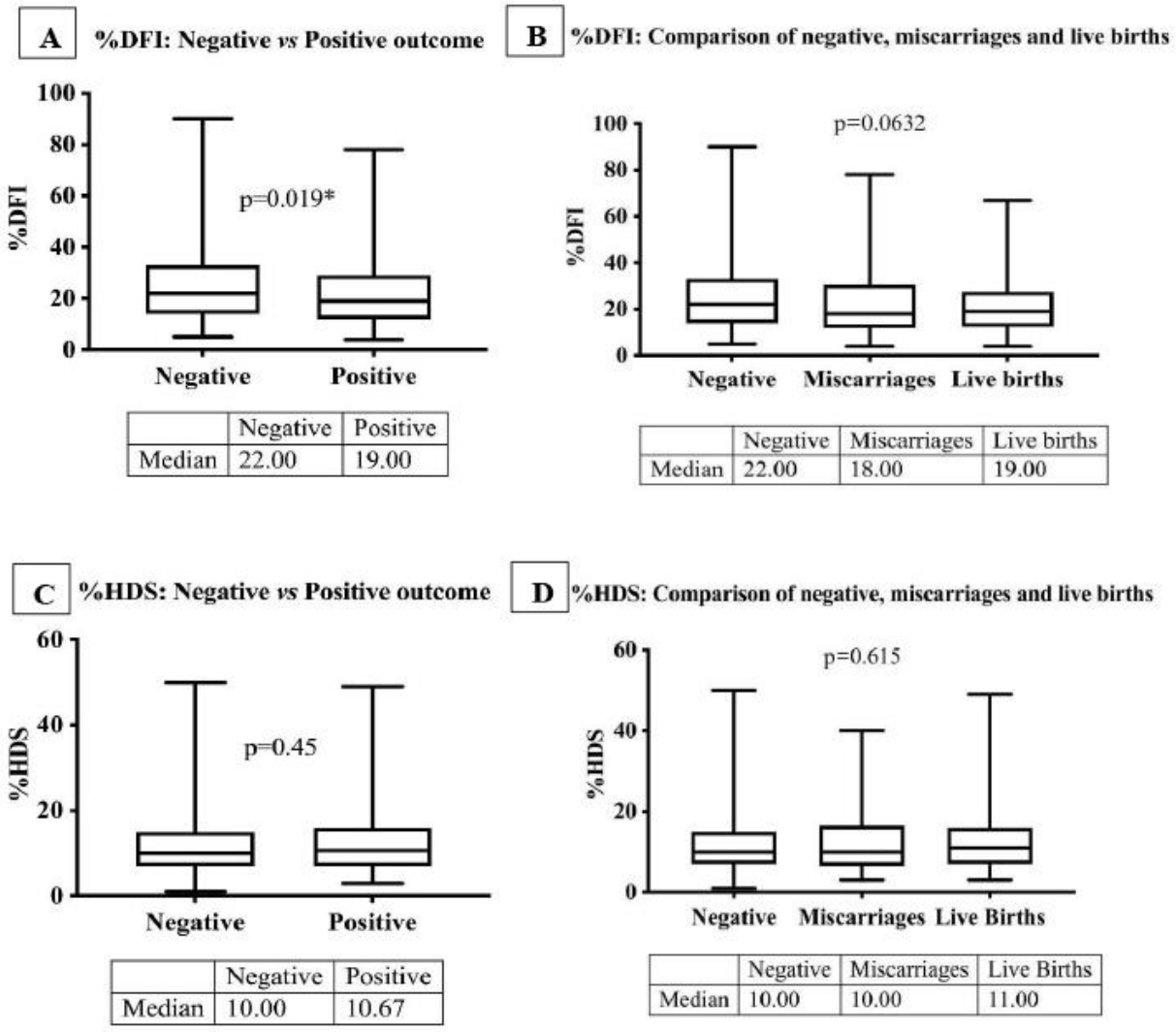


Figure 4: A) Sperm motility in pregnancy outcomes B) Male/ female factors and pregnancy outcome

We applied the Chi-square test to determine if there are differences in pregnancy outcomes based on whether patients are considered as "normal" or "asthenozoospermia", to reveal no statistically significant differences in pregnancy outcomes based on sperm motility (p=0.82) [Figure 4A].

We next aimed to understand how pregnancy outcomes were distributed among patients based on the presence of either male or female factors that might influence fertility. Our Chi-square analysis indicated that there were no notable differences in the distribution of patients among these categories. The calculated p-value of 0.78 confirmed that the proportions of patients with different pregnancy outcomes, stratified by the presence of male or female factors, did not show any significant differences [Figure 4B].

Finally, the %DFI and %HDS were correlated to pregnancy outcomes [Figure 5A-D]. A comparison of %DFI between patients achieving a positive pregnancy or negative pregnancy outcome post-ART procedure was performed. A column graph with the median %DFI values of the two groups is shown below. Statistical analyses using Mann-Whitney U-test indicated that the %DFI was significantly higher in patients with negative pregnancy outcomes (p=0.019*) [Figure 5A]. The %DFI in patients with a negative pregnancy outcome following an ART cycle was compared to patients with a positive pregnancy outcome resulting in miscarriages or live births utilizing the Kruskal-Wallis test. Median levels of the %DFI are indicated between the different groups (p=0.063) [Figure 5B]. A comparison of %HDS between patients achieving a positive pregnancy or negative pregnancy outcome post-ART procedure was performed. The negative group consisted of 324 patients and the positive group had 250 patients.



	Negative	Positive
Median	22.00	19.00

	Negative	Miscarriages	Live births
Median	22.00	18.00	19.00

	Negative	Positive
Median	10.00	10.67

	Negative	Miscarriages	Live Births
Median	10.00	10.00	11.00

Figure 5 (A-D): Percent DFI is significantly higher in patients with negative pregnancy outcome

A column graph with the median %HDS values of the two groups is shown below. Statistical analyses using Mann-Whitney U-test indicated that the %HDS was similar between the groups (p=0.45) [Figure 5C]. The %HDS in patients with a negative pregnancy outcome following an ART cycle was compared to patients with a positive pregnancy outcome resulting in miscarriages or live births utilizing the Kruskal-Wallis test. The results indicated that %HDS in the negative outcome group was not significantly different when compared to the miscarriage or live births group (p=0.615) [Figure 5D].

Discussion

Our comprehensive analysis of various factors influencing pregnancy outcomes in the context of ART procedures has provided valuable insights into the intricate dynamics of fertility. The examination of sperm count, a fundamental parameter in male fertility, did not reveal significant differences between patients who achieved positive pregnancy outcomes and those who did not. This observation aligns with the notion that sperm count alone may not be a robust predictor of pregnancy success. Similarly, the assessment of both male and female ages failed to demonstrate significant associations with pregnancy outcomes, emphasizing the multifactorial nature of fertility. Age plays a role in natural conceptions, however, in cases where fertility treatments have been involved, the selection of the best sperm/oocyte/embryo further increases the chances of a positive pregnancy outcome.

In our analysis of 574 couples undergoing ART treatments, the lack of statistical significance between male and female age and pregnancy outcomes may be attributed to several factors unique to our cohort. Firstly, the relatively narrow age range of participants could minimize age-related variability in outcomes. Additionally, our cohort might have had mitigating factors, such as higher overall health status or access to advanced fertility treatments, which can lessen the impact of age on ART success. It is also likely that the influence of age was overshadowed by other determinants of fertility not captured in our dataset, such as genetic factors, lifestyle choices, or environmental exposures. This unexpected finding underscores the complexity of fertility and the multifactorial nature of ART success, suggesting that age may not always be a predominant factor in all populations. Further research is warranted to explore these nuances in different cohorts.

Furthermore, the analysis of sperm motility and its impact on pregnancy outcomes did not yield statistically significant results, corroborating the idea that other factors, such as sperm morphology or genetic factors, may play a more prominent role. However, our investigation into %DFI revealed intriguing findings. A significantly higher %DFI was observed in patients who experienced negative pregnancy outcomes, highlighting a potential association between increased DNA fragmentation and reduced pregnancy success. This observation aligns with previous research that has underscored the relevance of %DFI as a predictor of ART outcomes and specifically, the utility of the gold standard SCSA® test. While %HDS values were also assessed, no significant differences emerged, suggesting that the type of DNA damage assessed may be a crucial factor in its predictive value.

Conclusion

In conclusion, our study underscores the complexity of factors influencing pregnancy outcomes in ART procedures. While sperm count and age did not emerge as significant predictors, the assessment of sperm DNA fragmentation, particularly %DFI, warrants further exploration as a potential prognostic marker. These findings, which are the largest to date and focus on the infertility population in the Indian sub-continent highlight the need for a more comprehensive

understanding of the multifaceted aspects of fertility and the continued exploration of novel biomarkers and factors that may enhance our ability to predict and optimize pregnancy outcomes in ART procedures.

Ethical Approval: Nil

Conflict of Interest: Nil

Financial Disclosure: None

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