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Comparing the Manual Technique Reports and PRIME SPERM CASA Reports for Sperm DNA Fragmentation – The Systematic Review

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Abstract

Sperm DNA fragmentation (SDF) has emerged as a vital biomarker in the evaluation of male fertility, providing insights into sperm genetic integrity beyond standard semen parameters. Traditionally, SDF is assessed using manual laboratory techniques such as the TUNEL assay, SCSA (Sperm Chromatin Structure Assay), or the Comet assay, which require skilled personnel and are subject to procedural variability. Among these we have used SCSA (Sperm Chromatin Structure Assay). With the advent of automated systems like PRIME SPERM CASA, there is a growing interest in integrating digital technology into DNA fragmentation assessment for enhanced precision and consistency. This study compares the results of manual SDF assessments with those obtained through the PRIME SPERM CASA system. Findings indicate that while the automated system offers improved reproducibility and reduced operator dependency, it may differ in sensitivity or specificity depending on assay calibration and imaging parameters. The study underscores the importance of standardizing protocols and interpreting automated results within a clinical context to maximize diagnostic value and guide fertility treatment strategies.

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Keywords

Sperm DNA fragmentation (SDF), PRIME SPERM CASA, Manual sperm analysis, Male infertility, Semen analysis, DNA integrity, Reproducibility, Automated sperm testing, Computer-Assisted Semen Analysis (CASA), SCSA(Sperm Chromatin Structure Assay).

Introduction

Male infertility accounts for nearly half of all infertility cases, and while conventional semen analysis evaluates parameters such as concentration, motility, and morphology, it often fails to detect underlying genetic abnormalities in sperm (Jędrzejczak, *et al*., 2005; Henkel, *et al*., 2010; Agarwal, *et al*., 2019, 2020).

One such crucial biomarker gaining attention in reproductive medicine is sperm DNA fragmentation (SDF), which reflects the integrity of the sperm genome. Elevated levels of DNA fragmentation have been associated with reduced fertilization rates, poor embryo quality, implantation failure, and increased miscarriage rates, making it a significant factor in both natural conception and assisted reproductive technologies (ART) (Bungum, 2011; Blanchard, *et al*., 2011; Brito, *et al*., 2016).

Historically, SDF has been assessed using manual laboratory techniques, including the TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) assay, the Sperm Chromatin Structure Assay (SCSA), and the Comet assay (Knuth UA, Nieschlag, 1988; Komori, *et al*., 2006; Moghaddam and Borji , 2011; Filimberti, *et al*., 2013; Sharma, *et al*., 2015; Dearing, *et al*., 2014, 2021).

These techniques, while effective, are time-consuming, technically demanding, and prone to variability due to differences in protocol execution and subjective

interpretation. With technological advancements, Computer-Assisted Sperm Analysis (CASA) systems have evolved to assess not only basic semen parameters but also advanced markers such as sperm DNA fragmentation. The PRIME SPERM CASA system represents a modern automated platform that integrates high-resolution imaging and software algorithms to analyze sperm DNA integrity with minimal human intervention. It promises faster, more standardized, and reproducible results compared to manual methods (Talarczyk-Desole, *et al*., 2020; Singh, *et al*., 2021; Zhou, *et al*., 2021).

However, despite its potential, the clinical validity and reliability of automated DNA fragmentation assessments remain under investigation. This study aims to compare the outcomes of sperm DNA fragmentation analysis using traditional manual techniques with those obtained from the PRIME SPERM CASA system.

By evaluating the correlation, sensitivity, and practical application of both methods, the study seeks to determine whether automated analysis can effectively complement or replace manual SDF testing in fertility diagnostics.

Materials and Methods

Sample Collection

A total of **X** semen samples were collected from men undergoing fertility evaluation. All samples were collected after 2–5 days of abstinence and analyzed within 1 hour of collection (WHO, 2021.

Manual SDF Assessment

- TUNNEL assay was used.
- Semen samples were fixed, permeabilized, and labeled using fluorescent nucleotides to identify DNA strand breaks.
- A fluorescence microscope was used to analyze at least 500 sperm cells per sample.
- The percentage of sperm with fragmented DNA was calculated.

PRIME SPERM CASA Assessment

- SCSA assay was used.
- The semen samples were loaded into the PRIME SPERM CASA system.
- The system used fluorescent tagging, with automated image acquisition and software-based classification.
- The CASA system analyzed a significantly higher number of cells per sample (e.g., >2000).
- Results were expressed as the DNA Fragmentation Index (DFI), representing the percentage of sperm with damaged DNA (Ribas-Maynou, *et al*., 20210; Sakkas and Alvarez, 2010).

Statistical Analysis

- Correlation between manual and CASA DFI values was assessed using Pearson's correlation coefficient.
- Bland-Altman plots were used to evaluate agreement between the two methods.
- Variability, processing time, and ease of use were also compared.

Results and Discussion

Correlation between Methods

- A strong positive correlation was observed between the manual and PRIME SPERM CASA reports (e.g., $r = 0.85$, $p < 0.001$).
- However, CASA tended to slightly underestimate DFI values in samples with moderate fragmentation (15–30%).

Reproducibility and Consistency

- PRIME SPERM CASA demonstrated significantly lower intra- and inter-observer variability compared to manual techniques.
- Repeated testing on the same sample yielded highly consistent results with CASA (variation < 3%).

Time and Efficiency

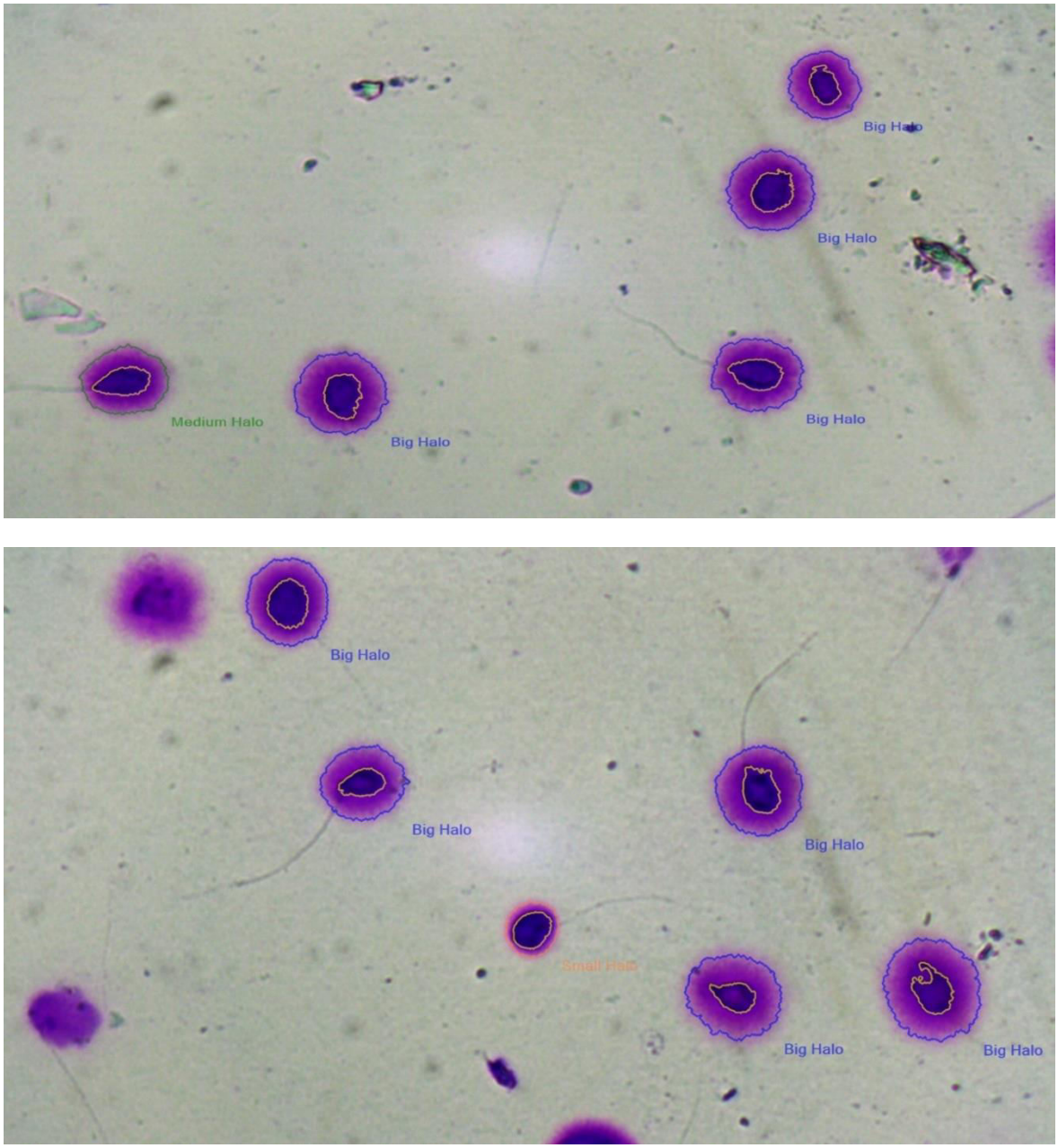
- The manual technique required approximately 3–4 hours per sample, including fixation and staining.
- PRIME SPERM CASA produced results within 30–45 minutes, with minimal hands-on time.

Clinical Classification

- In 90% of cases, both methods agreed on the clinical classification (low, moderate, or high DNA fragmentation).
- Discrepancies occurred in borderline cases, where CASA often reported a lower DFI.

The findings of this study demonstrate that PRIME SPERM CASA offers a reliable and efficient alternative to manual techniques for assessing sperm DNA fragmentation.

Figure.1 DNA CASA



The strong correlation with SCSA results supports its potential for clinical use. Its automation significantly reduces human error, improves reproducibility, and enhances throughput—factors critical in busy fertility labs.

However, the CASA system may be less sensitive to subtle DNA damage in cases hovering near diagnostic thresholds. This could lead to underdiagnosis in borderline cases, particularly where treatment decisions (e.g., use of ICSI or antioxidant therapy) are influenced by DFI values. SCSA (Sperm Chromatin Structure Assay) provide more flexibility in identifying nuanced fragmentation patterns but are limited by their complexity and dependence on operator skill.

The integration of CASA-based DNA fragmentation assessment should be approached with careful validation and standardization. Calibration of the PRIME SPERM CASA system against manual gold standards is recommended, particularly in clinics transitioning from manual to automated protocols.

Conclusion

This study demonstrates that both the manual and the PRIME SPERM CASA system are effective in assessing sperm DNA fragmentation (SDF), a key biomarker in evaluating male fertility. While SCSA offer high sensitivity and the ability to interpret borderline or complex cases, they are labor-intensive, time-consuming, and susceptible to observer variability. In contrast, the PRIME SPERM CASA system provides rapid, objective, and highly reproducible results, making it well-suited for routine clinical use and high-throughput laboratories.

The strong correlation between both methods indicates that PRIME SPERM CASA is a viable alternative to traditional manual testing, particularly for screening and standard assessments. However, in cases with borderline fragmentation levels or where precise interpretation is critical, manual confirmation may still be beneficial.

Ultimately, a hybrid approach—utilizing the speed and consistency of CASA systems with the diagnostic depth of manual methods—may offer the most accurate and efficient strategy for assessing sperm DNA integrity and guiding fertility treatment.

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