Relationships Among the Clinical Results of Women Undergoing IUI and Increased Basal Sperm DNA Fragmentation

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Abstract:
Background: The use of sperm DNA fragmentation (SDF) test to assess male infertility is growing. Purpose: To investigate how women going through IUI are influenced clinically through the sperm DNA fragmentation index (DFI). Methods: This retrospective research was carried out at (Qena center for IVF and ICSI) from July 2023 to July 2025. Results: There were none statistically significant variances among the normal sperm DFI group as well as abnormal sperm DFI group regarding the biochemical rate of pregnancy (21.89% vs. 19.23%, p-value equal to 0.370), clinical pregnancy rate (18.97% vs. 15.38%, p-value equal to 0.430), delivery rate (17.5% vs. 11.5%, p-value equal to 0.321), live birth rate (17.15% vs. 11.5%, p-value equal to 0.3), or pregnancy loss rate (34.0% vs. 33.3%, p-value equal to 1.000). Area under the Curve (AUC) was 0.56, suggesting a low discriminative power of Sperm DNA fragmentation index in predicting IUI pregnancy results. Conclusions: Sperm DNA fragmentation alone was not a strong distinct indicator of the achievement of IUI and ought to be understood alongside additional semen as well as female factors in healthcare practice.

Keywords: DNA fragmentation, Intrauterine insemination, Pregnancy, Semen.

1. Introduction

The approximated frequency rate of infertility is between ten percent and fifteen percent, making it a major global health concern. Just one out of every seven married couples is thought to struggle sense of being unfulfilled desire to conceive for longer than a year (Huang et al., 2024).

In comparison to other assisted reproductive techniques, intrauterine insemination (IUI) is regarded as the initial-line technique for infertile or hypo-fertile couples because of its uncomplicated nature of management, low rate of adverse effects, in addition to affordable cost (Ahmed et al., 2017).

Since damage to the sperm chromatin is thought to be linked to an inadequate reproduction response, SDF, or damage in the male germ line in the form of single- or double-strand breaks happening at the testicular, epididymal, or post-ejaculatory levels, has drawn a lot of attention across the years (Aitken, 2017) and was suggested as a potential test for evaluating sperm function (Sugihara et al., 2022). SDF is only partially correlated with semen quality (Punjabi et al., 2018) and has risen in subfertile men (Punjabi et al., 2019) than in the fertile group.

The great majority of research on SDF had concentrated on results of vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) (Cissen et al., 2016; Simon et al., 2017). Additionally, SDF was linked to higher miscarriage frequency as well as decreased pregnancy rates for (ICSI) and (IVF) (Zhao et al., 2014; Zini et al., 2011). Although there is still debate, sperm DNA fragmentation has been proposed as a predictor of (IUI) pregnancy. Inadequate reproduction results for individuals going through IUI were linked to increase SDF (Chen et al., 2019).

The present study aimed to investigate how women going through IUI are affected clinically by the sperm DNA fragmentation index.

2. Patients and methods:

This retrospective research has been performed at (Qena center for IVF and ICSI) from July 2023 to July 2025. All participants in the research provided written informed consent. Ethical approval has been also attained.

A total of 900 male semen samples had been gathered to treat 300 IUI cycles. Inclusion criteria involved all the prior tests performed by infertile couples, without chromosomal anomalies, and the woman's fallopian tubes had been free of obstruction (a minimum of one of them side). The women had ovulation along with prevailing follicle growth, mild oligospermia, male sex problems, as well as other conditions.

Depending on their sperm DFI levels, men have been classified into 2 groups: the normal sperm DFI group (DNA fragmentation index less than thirty percent) as well as the abnormal sperm group (DNA fragmentation index equal or more than 30%) (Bungum et al., 2007). Patients' baseline characteristics such as body mass index (BMI), age, length of infertility, etc., were gathered.

Infertile females had consistent menstruation as well as regular ovulation underwent IUI in their natural cycles. Ingestion of letrozole (LE) or clomiphene (CC) either alone or paired with gonadotropins or gonadotropins alone have been employed to motivate ovaries to trigger ovulation in patients with ovulation problems, unusual follicles growth, or extended menstrual cycles following vaginal ultrasound testing on the 3rd to 5th day of the menstrual cycle. Vaginal B-ultrasound was used to continuously observe follicle development starting on eighth day of the menstrual cycle. IUI was performed 36 to 42 hours following 5,000–10,000 IU of human chorionic gonadotropin (hCG) have been administered injections via the muscle to trigger ovulation once follicles had a measurement of roughly eighteen mm emerged.

Masturbation was used for gathering sperm while the men abstained for two to seven days. The Laboratory Manual for Human Semen Examination and Processing, Fifth Edition (World Health Organization, 2010) was followed when performing standard semen preparation and assessment. A computer-aided semen analyser was used to assess as well as document the overall quality of the semen.

3. Sperm DNA fragmentation index

The sperm chromatin analysis (SCSA) kit (Name of company) has been utilized to conduct the sperm DNA fragmentation assay (SDFA) strictly in compliance with the company's guidelines (Evenson et al., 2020). The following was the extensive evaluation procedure. Initially 0.1 milliliters of solution A (TNE buffer, sperm dilution) was combined with a suitable amount of semen. Then, 0.2 milliliters of solution B (acid solution of 0.1 percent Triton X-100, 0.15 mol/L NaCl, and 0.08 mol/L HCl, pH 1.2) has been added and mixed. 0.6 milliliters of acridine orange (AO) staining solution (six micrograms per milliliter AO, 37 mmol/L citric acid, 126 mmol/L Na2HPO4, 1 mmol/L Na2EDTA, 0.15 mol/L NaCl, pH 6.0) has been added and combined after the mixture had stood for thirty seconds. A flow cytometer was used to identify the sperm DFI following a three-minute staining period. The software (….) was used to analyze the data after at least 5,000 sperm were collected. The total percentage of DNA fragmentation is medium + high. The proportion of sperm with fragmented DNA relative to the total amount of sperm was used for expressing the sperm DFI. The replicate DFI measures showed fewer than five percent variance.

4. Optimizing semen for IUI

Subsequently two to seven days of abstinence, the semen had been gathered two hours before intrauterine insemination (IUI). It was kept in a sterilised extractor as well as allowed to liquefy at 37°C. Centrifugation using density gradients was performed on semen utilizing eighty percent as well as forty percent SpermGrad solutions that had been heated to 37°C. Layering the two gradient media in a centrifuge tube, incorporating liquefied semen, as well as modifying volumes regarding the sperm conditions were all steps in the process. Following a fifteen-minute centrifugation at 300–400 × g, sperm pellet had been removed, combined via insemination solution, along with centrifuged once more. Sperm suspension was subsequently determined for application in IUI after the supernatant had been eliminated, as well as the sperm DNA fragmentation index (SDFI) was measured before the preparation.

5. Intrauterine insemination (IUI)

Following bladder emptying as well as cleaning vulva by regular saline, patient had been in the position of lithotomy. A cotton swab utilized to wipe vagina, cervix, and fornix. After connecting one ml syringe to a non-reusable assisted reproduction tube, 0.5 ml of sperm suspension had been delicately as well as gradually inserted by means of cervix as well as approximately one centimeter above cavity of uterus. Assisted reproduction tube was further eventually eliminated shortly after a brief stay. Following the procedure, the patient was told to elevate the hip at an angle of thirty degrees for 15 to 30 minutes while being observed in bed. If there was no particular sensation of uncomfortable, the patient was given instructions to depart.

6. Monitoring the results of pregnancy

Fourteen to sixteen days following IUI, a blood test was conducted to assess the β-hCG level in peripheral blood to figure out if biochemical pregnancy (defined as more than 5.0 mIU/ml) had been detected. Vaginal ultrasonography had been utilized to view intrauterine pregnancy sac across fourth and fifth weeks after IUI. Within stimulation cycles, luteal assistance (20 mg progesterone consumed orally every day) was administered starting 48 hours following IUI and continuing till clinical pregnancy if β-HCG was positive. A miscarriage, ectopic pregnancy, and stillbirth were pregnancy loss examples (Duffy et al., 2021).

7. Statistical analysis

Recorded information was analyzed utilizing the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative information was represented as mean± standard deviation and ranges when their distribution was parametric (normal) whereas non-normally distributed parameters (non-parametric information) was represented as median with inter-quartile range (IQR). Also qualitative parameters were represented as number and percentages. Information was explored for normality utilizing Kolmogorov-Smirnov and Shapiro-Wilk Test.

8. Results:

The results of the current study are displayed in the following tables:

Among 300 cycles of IUI, 274 were normal sperm DFI cases (Group A), as well as 26 of abnormal sperm DFI (Group B). As demonstrated in Table (1); the mean age of male in Group A was 30.29 ± 11.57 and 30.86 ± 11.17 years in Group B with none substantial distinction among both groups (p=0.647); the mean female age in Group A was 25.64 ± 4.36 and 26.96 ± 6.13 years in Group B with none substantial distinction among both groups (p=0.239); the mean BMI of male in Group A was 25.35 ± 2.97 and 25.76 ± 1.89 kg/m2 in Group B with none substantial distinction among both groups (p=0.161); the mean duration of infertility in Group A was 3.54 ± 1.03 and 4.28 ± 0.91 years in the Group B with substantial distinction among both groups (p=0.001); the mean Sperm DFI% in Group A was 13.18 ± 1.53 and 40.12 ± 16.66 in Group B with highly substantial distinction among both groups (p<0.001);); mean Sperm high DNA stainability in Group A was 6.66 ± 1.91 and 7.67 ± 1.78 in Group B with none substantial distinction among both groups (p=0.145).

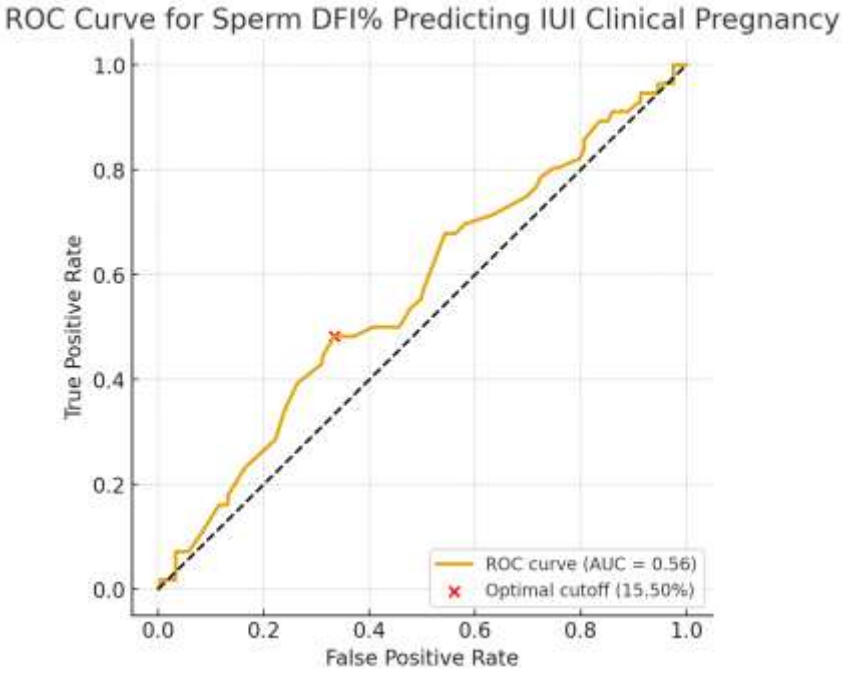
Table (1) Baseline characteristics of the study groups

	Group A (DFI<30%) (n=274)	Group B (DFI≥30%) (n=26)	p-value
Male age (years)	30.29 ± 11.57	30.86 ± 11.17	0.647
Female age (years)	25.64 ± 4.36	26.96 ± 6.13	0.239
BMI of male (kg/m^2)	25.35 ± 2.97	25.76 ± 1.89	0.161
Duration of infertility (years)	3.54 ± 1.03	4.28 ± 0.91	0.001
Sperm DFI%	13.18 ± 1.53	40.12 ± 16.66	<0.001
Sperm high DNA stainability	6.66 ± 1.91	7.67 ± 1.78	0.145

Table (2) showed none substantial distinction among Group A and Group B regarding the rate of biochemical pregnancy (21.89% versus 19.23%, p = 0.370), clinical pregnancy frequency (18.97% versus 15.38%, p = 0.430), delivery rate (17.5% versus 11.5%, p = 0.321), live birth rate (17.15% versus 11.5%, p-value equal to 0.3), or pregnancy loss rate (34.0% versus 33.3%, p = 1.000)

Table (2) Clinical outcomes in intrauterine insemination cycles

	Group A (DFI<30%) (n=274)	Group B (DFI≥30%) (n=26)	p-value
Biochemical pregnancy	60/274 (21.89%)	5/26 (19.23%)	0.370
Clinical pregnancy	52/274 (18.97%)	4/26 (15.38%)	0.430
Delivery rate	48/274 (17.5%)	3/26 (11.5%)	0.321
Live birth rate	47/274 (17.15%)	3/26 (11.5%)	0.3
Pregnancy loss rate	16/47 (34.0%)	1/3 (33.3%)	1.000



The ROC curve above shows the predictive ability of **(DFI %)** for **clinical pregnancy after IUI**.
Area under Curve (AUC) was **0.56**, suggesting a **low discriminatory authority** of Sperm DFI in the prediction of IUI consequences of pregnancy — only slightly better than chance.

The **optimal cutoff value** recognized by **Youden index** was around **15.5% DFI**, giving:
Sensitivity (rate of true positive) ≈ 48%,
Specificity (rate of true negative) ≈ 67%.

Interpretation:
Couples had sperm DFI below around **15.5%** had a somewhat higher likelihood of achieving pregnancy following IUI contrasted with others above that threshold, but the difference is modest. The low AUC indicates that sperm DF alone is **not a strong independent predictor** of IUI success as well as ought to be understood alongside additional semen as well as female factors in clinical practice.

9. Discussion:
To standardize the selection of SDF assays and establish the proper DFI cutoff values, high-quality clinical investigations are crucial. In addition, advances in assay degree of complexity, subjective nature, as well as affordability will make SDF testing more accessible (Adler et al., 2023).

The correlation among sperm DFI as well as clinical outcomes in 300 IUI cycles was examined in this work. They had been separated into 2 groups depend on the sperm DFI diagnostic criteria: 274 of the cases were normal (91.33%) and 26 cases were abnormal (8.67%). The statistical analysis outcomes revealed nonsubstantial distinction among Group A (DFI<30%) and Group B (DFI≥30%) as regards male age, female age, BMI of male, and sperm high DNA stainability ($p > 0.05$). While there was substantial distinction among both groups (p-value equal to 0.001) regarding infertility length period and highly substantial distinction among both groups ($p=0.001$) regarding sperm DFI% ($p<0.001$). These results agreed with Yang et al., (2019) who also documented none substantial distinction among the normal DFI and abnormal DFI groups concerning male age, female age, male BMI also they found highly substantial distinction among both groups (p-value equal to 0.001) regarding sperm DFI% ($p<0.001$). Our findings disagreed with Zhu et al., (2022), they noted substantial distinction among both groups ($p=0.022$) regarding male age and none substantial distinction among both groups concerning infertility duration, as well as sperm DFI%.

Many researchers believed the concept SDF negatively affects embryos quality as well as outcomes of pregnancies following IVF/ICSI (Virro et al., 2004; Bounartzi et al., 2016). Compared to people with low DFI, those with raised DFI had much increased frequencies of pregnancy as well as early abortions (Bounartzi et al., 2016).

The current work results revealed none substantial distinction among the normal sperm DFI group and the abnormal sperm DFI group regarding the biochemical pregnancy rate (p-value equal to 0.370), clinical pregnancy rate (p-value equal to 0.430), delivery rate (p-value equal to 0.321), live birth rate (p-value equal to 0.3), or pregnancy loss rate ($p = 1.000$). These findings were consistent with Zhu et al., (2022), they found demonstrated that IUI clinical outcomes were not substantially affected by raised basal sperm DFI. There were no differences in the pregnancy rates after intrauterine insemination among low, medium and high DFI groups in Yang et al., (2019) study.

For assessing how well sperm DFI predicts IUI clinical results, (ROC) curves were created.

Our results revealed that **(AUC)** was **0.56**, suggesting a **low discriminative power** of Sperm DNA fragmentation index in predicting IUI pregnancy results. These findings also were supported by Zhu et al., (2022), the sperm DNA fragmentation index wasn't useful in anticipating pregnancy for cases following intrauterine insemination, and regarding ROC curves (all areas under the ROC curve% of clinical results less than fifty-four percent).

Another research conducted by Yu et al., (2025), revealed that Sperm DFI's AUC values was varied from 0.568 to 0.706, suggesting that it was not very useful as a stand-alone predictive test. These results imply that sperm DNA fragmentation index by itself might not be a very good independent predictor of recurrent spontaneous abortion (RSA) (Jiang et al., 2025).

10. In conclusion,

IUI clinical outcomes may not be well predicted by sperm DFI alone.

References:

1. Adler, A., Roth, B., Lundy, S. D., Takeshima, T., Yumura, Y., & Kuroda, S. (2023). Sperm DNA fragmentation testing in clinical management of reproductive medicine. Reproductive medicine and biology, 22(1), e12547.
2. Ahmed, B., Gowri, V., Silja, A., AlSabti, J., Al-Khaduri, M., Pathare, A. (2017). Factors influencing the success Rate of intrauterine insemination: A retrospective study in Sultan Qaboos University Hospital. J. Women's Health Care. 6:1000402.
3. Aitken, R.J. DNA damage in human spermatozoa; Important contributor to mutagenesis in the offspring. Transl. Androl. Urol. 2017; 6((Suppl. 4)): S761–S764.
4. Bounartzi, T., Dafopoulos, K., Anifandis, G., Messini, C. I., Koutsonikou, C., Kouris, S., Satra, M., Sotiriou, S., Vamvakopoulos, N., & Messinis, I. E. (2016). Pregnancy prediction by free sperm DNA and sperm DNA fragmentation in semen specimens of IVF/ICSI-ET patients. Human fertility (Cambridge, England), 19(1), 56–62.
5. Bungum, M., Humaidan, P., Axmon, A., Spano, M., Bungum, L., Erenpreiss, J., & Giwercman, A. (2007). Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Human reproduction (Oxford, England), 22(1), 174–179.
6. Chen, Q., Zhao, J. Y., Xue, X., & Zhu, G. X. (2019). The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta-analysis. Reproductive toxicology (Elmsford, N.Y.), 86, 50–55.
7. Cissen, M., Wely, M.V., Scholten, I., Mansell, S., Bruin, J.P., Mol, B.W., Braat, D., Repping, S., Hamer, G. (2016). Measuring Sperm DNA Fragmentation and Clinical Outcomes of Medically Assisted Reproduction: A Systematic Review and Meta-Analysis. PLoS ONE. 11: e0165125.
8. Duffy, J. M. N., Bhattacharya, S., Bhattacharya, S., Bofill, M., Collura, B., Curtis, C., Evers, J. L. H., Giudice, L. C., Farquharson, R. G., Franik, S., Hickey, M., Hull, M. L., Jordan, V., Khalaf, Y., Legro, R. S., Lensen, S., Mavrelos, D., Mol, B. W., Niederberger, C., Ng, E. H. Y., … Core Outcome Measure for Infertility Trials (COMMIT) initiative (2021). Standardizing definitions and reporting guidelines for the infertility core outcome set: an international consensus development study. Fertility and sterility, 115(1), 201–212.

9. Evenson, D. P., Djira, G., Kasperson, K., & Christianson, J. (2020). Relationships between the age of 25,445 men attending infertility clinics and sperm chromatin structure assay (SCSA®) defined sperm DNA and chromatin integrity. Fertility and sterility, 114(2), 311–320.
10. Huang, C., Shi, Q., Xing, J., Yan, Y., Shen, X., Shan, H., Sun, H., & Mei, J. (2024). The relationship between duration of infertility and clinical outcomes of intrauterine insemination for younger women: a retrospective clinical study. BMC pregnancy and childbirth, 24(1), 199.
11. Jiang, H., Xia, X., Luo, Y., Pan, H., Qu, S., & Xu, J. (2025). Sperm DNA fragmentation index: limited effectiveness on predicting embryo quality in assisted reproduction technology treatments. Reproductive biology and endocrinology: RB&E, 23(1), 14.
12. Punjabi, U., Van Mulders, H., Goovaerts, I., Peeters, K., Clasen, K., Janssens, P., Zemtsova, O., & De Neubourg, D. (2018). Sperm DNA fragmentation in the total and vital fractions before and after density gradient centrifugation: Significance in male fertility diagnosis. Clinical biochemistry, 62, 47–54.
13. Punjabi, U., Van Mulders, H., Goovaerts, I., Peeters, K., Roelant, E., & De Neubourg, D. (2019). DNA fragmentation in concert with the simultaneous assessment of cell viability in a subfertile population: establishing thresholds of normality both before and after density gradient centrifugation. Journal of assisted reproduction and genetics, 36(7), 1413–1421.
14. Simon, L., Zini A., Dyachenko, A., Ciampi, A., Carrell, D.T. (2017). A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J. Androl. 19:80–90.
15. Sugihara, A., Punjabi, U., Roelant, E., & De Neubourg, D. (2022). Is There a Relationship between Sperm DNA Fragmentation and Intra-Uterine Insemination Outcome in Couples with Unexplained or Mild Male Infertility? Results from the ID-Trial. Life (Basel, Switzerland), 13(1), 11.
16. Virro, M. R., Larson-Cook, K. L., & Evenson, D. P. (2004). Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertility and sterility, 81(5), 1289–1295.
17. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Geneva: World Health Organization; (2010).
18. Yang, H., Li, G., Jin, H., Guo, Y., & Sun, Y. (2019). The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. Translational andrology and urology, 8(4), 356–365.
19. Yang, H., Li, G., Jin, H., Guo, Y., & Sun, Y. (2019). The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. Translational andrology and urology, 8(4), 356–365.
20. Yu, H., Shi, C., Zhan, C., Wang, C., & Chen, J. (2025). Association of sperm DNA fragmentation with higher miscarriage rates in non-male factor infertility reproductive cycles. Translational andrology and urology, 14(5), 1456–1465.
21. Zhao, J., Zhang, Q., Wang, Y., & Li, Y. (2014). Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis.

Fertility and sterility, 102(4), 998–1005.e8.
22. Zhu, C., Zhang, S., Chen, F., She, H., Ju, Y., Wen, X., Ji, Y., Pan, Y., Yang, C., Sun, Y., Dong, N., Liu, K., Li, F., Xue, T., & Cui, H. (2022). Correlations between elevated basal sperm DNA fragmentation and the clinical outcomes in women undergoing IUI. Frontiers in endocrinology, 13, 987812.
23. Zini A. (2011). Are sperm chromatin and DNA defects relevant in the clinic? Systems biology in reproductive medicine, 57(1-2), 78–85.