International Journal of Scientific Research in Modern Science and Technology, ISSN: 2583-7605 (Online)
Vol. 1, Issue 1 [2022] www.ijsrmst.com

A generalized study of human sperm DNA fragmentation between Asthenozoospermia and Normozoospermia semen sample

Amandeep Singh[1], Ram Dayal[2*], Deepika Verma[3], Jeetendera Verma[4], Kamla Singh[2], Ramesh Kumar Gupta[5]

[1]Master Scholar in Clinical Embryology & Pre-Implantation Genetic, ASPIER, Mysore, Karnataka
[2]Institute of Reproduction and Child Cares & IRCC IVF Centre, Panchkula, Haryana
[3]UG Scholar (B.Sc. Medical), Sector-14, Government Postgraduate College for Women, Panchkula, HR, INDIA
[4]PG Scholar Zoology, ANDK P.G. College, Babhnan, Gonda, Uttar Pradesh, INDIA
[5]Trainee Embryologist at IRCC IVF Centre, Sector 17, Panchkula, Haryana, INDIA

Corresponding Author Email id: ramdayal2509@gmail.com[2*]

Abstract:

Objective: A generalized study of human sperm DNA fragmentation between Asthenozoospermia and Normozoospermia semen sample. **Design:** An observational study of human sperm DNA fragmentation in semen samples carried out during my master's dissertation. **Settings:** Institute of Reproduction and Child Cares & IRCC IVF Centre, Panchkula, Haryana. **Patient Selection:** All male infertility patients visited for semen analysis. Those patients whose consent was not obtained weren't included in this study, and Azoospermic patients were excluded as well. **Duration of Study:** 3 Months. **Outcome:** Sperm DNA fragmentation percentage was evaluated in Asthenozoospermia and Normozoospermia and recorded differences. **Results:** In this observational research, 31 patients were included who had semen analysis for infertility therapy at the Institute of Reproduction and Child Cares & IVF Center between March 2021 and May 2021.20 of the individuals (64.51%) were taken into consideration for the sperm DNA fragmentation test.Rest patients 11 (35.48 %) wasn't given consent for research purpose; we excluded those patients from this study. During Sperm DNA fragmentation evaluation, we found degraded (type a) was 3.7 %, without halo (type b) 4.28 %, Medium halo (type c) 11.73% and Big halo (type d) 5.29 % in AZS males and degraded (type a) was 2.34 %, without halo (type b) 3.89 %, Medium halo (type c) 45.54 % and Big halo (type d) 23.23 % in NZS males. **Conclusion:** The mean of sperm with Sperm DNA fragmentation (SDF) [degraded (a) + without halo (b) = 160.60%] was found in Asthenozoospermia, and the mean of sperm with SDF [degraded (a) + without halo (b) = 124.60%] in Normozoospermia.

Key words: Asthenozoospermia, DNA Fragmentation, Human Sperm, Normozoospermia Semen Preparation, Swim-up.

1. Introduction:

After 12 months of consistent, unprotected timed sexual activity, infertility is defined as the couple failing to conceive clinically [1]. Male factors alone or in combination with female variables impact more than 15% of couples worldwide, accounting for 50% of instances of infertility [2]. Male infertility is often assessed by a standard semen analysis, however semen analysis alone is insufficient to forecast male fertility potential and the efficacy of assisted reproductive technology (ART) [3]. Actually, a normal semen analysis is present in 15% of people who are infertile [4]. However, the evaluation of sperm concentration and motility may not accurately represent the real level of DNA fragmentation in sperm [5], which has a negative impact on proper fertilisation, embryonic development, and ART success [6].

Sperm DNA fragmentation (SDF) has been linked to oxidative stress, faulty sperm cell maturation, failed programmed cell death, and environmental contaminants in addition to heat exposure, smoking, environmental pollutants, and chemotherapy [7, 8, 9, 10]. Oxidative stress and male infertility are closely

related [12]. It is impossible to deny the function of reactive oxygen species in physiological processes including necrosis and capping, but an excess of ROS may have a number of negative consequences, including SDF [13].

DNA damage may take the form of mismatched bases, lost bases, changed bases, adducts and crosslinks, pyrimidine dimers, single strand breaks (SSBs), double strand breaks (DSBs), and pyrimidine dimers. But it's crucial to remember that some of these changes might result in SDF and jeopardise the effectiveness of ART or spontaneous conception.

Increased SDF also has a deleterious impact on pregnancy rates, as shown by several research [14]. According to scientific research, DNA damage in spermatozoa might have a bad effect on the wellbeing of the offspring. There will probably be an increase in the use of SDF testing by doctors in clinical settings because of SDF's detrimental effects on male fertility [15]. There has been investigation into a number of interferences in an effort to improve fertility outcomes and support strong progeny [16].

SDF is thought to be primarily brought on by oxidative stress in the male reproductive system, inadequate maturation and unsuccessful apoptosis in the testis, and other factors [17]. During spermatogenesis, transitional proteins and protamines are switched to create compact chromatin. The endogenous nuclease topoisomerase II breaks DNA to lessen torsional stress for histone disintegration and chromatin packing [18–20]. Human embryos have a self-repair mechanism for DNA, but if these breaks do not seem to be repaired, or chromatin packaging is impaired, it can lead to defects in maturation and the appearance of sperm through increased SDF in ejaculates [16]. Several studies have indicated that DNA fragmented spermatozoa can fertilize oocytes [21-22], but these spermatozoa are associated with abnormal embryo quality, block blastocyst development, and lower conceiving rates, whether they are conceived naturally or with assisted reproductive technologies. Sperm DNA damage has been linked to male infertility in an increasing number of research, according to this information. Fertilization and development of a healthy offspring are dependent on the fragmentation of sperm DNA [17]. This study provides evidence for its use in treating infertile men by comparing Asthenozoospermia with Normozoospermia in terms of sperm DNA fragmentation.

Material & Method:

March 2021 to May 2021 saw the completion of this broad investigation. In the Andrology Laboratory of the Institute of Reproduction and Child Cares & IRCC IVF Centre, Panchkula, Haryana, semen samples were collected from 31 men who had signed up for the semen analysis test. Patient advised to give semen sample after maintaining sexual abstinence of 2 – 7 days. Semen samples were collected and examined after liquefaction (30 - 60 minutes at 37 °C) from 31 patients. Patients were then classified as Asthenozoospermia [progressive sperm motility (a+b) < 32 %, n = 5), Normozoospermia [progressive motility $\geq 32\%$, n = 15].

Material requirements: we were used following material in this research:

1. Semen Collection container
2. Semen Sample
3. QwikCeck^{TM} DFI Kit [Medical Electronic Systems (INDIA) PVT. Ltd].
4. 80% Ethanol, 90% Ethanol and Absolute Ethanol 99.99 %
5. Distilled water
6. Phase contrast microscope
7. 3 ml BD Falcon sterile pipette
8. 200 micro liter pipette and pipette tips sterile
9. Refrigerator and Ice pack
10. Water bath
11. Digital incubator
12. SF800 Centrifuge machine
13. 22 x 22 mm cover slip
14. Cell counter
15. Semen sample preparation kit

1.1. Sperm DNA fragmentation method:

Human sperm DNA fragmentation was determined using a commercial QwikCheck DFI kit from Medical Electronic Systems (INDIA) PVT. Ltd]. By maintaining 20 million sperm count at 37 °C, swim-up sperms were mixed in melted agrose gel. DNA loops were dispersed throughout a halo of nonfragmented sperm DNA. There was a small or no halo of DNA dispersion around fragmented sperm DNA. It was observed that DNA fragmentation in Asthenozoospermia and Normozoospermia was performed in the following way:

1. First of all, we were kept the DFI kit at room temperature one-hour prior use.
2. Routine Semen Analysis
3. Semen preparation by density gradient
4. Swim-up at least 5 – 10 minutes
5. Again do the semen analysis in swim-up volume and maintain sperm count 20 x 10^6/ml
6. Melt the agrose gel in same tube at 80^0C for 6 minutes.
7. After melting the gel we were kept at 37^0C for 5 minutes.
8. We were adding 60 µl of diluted swim-up sperms in melted agrose gel at 37^0C.
9. We were take 20 µl of sperm and argose gel suspension on clean glass slide or coated glass slide.
10. Quickly place the cover slip carefully avoiding air bubble formation.
11. Immediately keep the slide 2-8 ^{0}C for 7 minutes.
12. After that remove cover slip carefully and keep the slide horizontal position.

13. Quickly add the denaturing solution (cover complete slide) and allow for 6 minutes at room temperature (RT).
14. After that remove the denaturing solution and add lysing solution (cover the slide completely) for 20 minutes at RT.
15. Remove the lysing solution and add abundant distilled water and allow for 5 minutes.
16. Remove distilled water and immediately add fixative 1 and allow for 2 minutes.
17. After that remove fixative 1 and add fixative 2 and allow for 2 minutes.
18. Remove fixative 2 and immediately add 6 drops of Giemsa A and allow for 1 minute.
19. After that add 12 drops of Giemsa B and allow for 2 minutes.
20. Gently wash the slide (or Note: no wash only wipe the slide back side with sterilium or 70 % alcohol).
21. Leave it for air dry at least 10 minutes.

2. Reporting of DFI:

We observed the DFI stained slide under the 40x objective lens and counted 500 sperms and noted as degraded (type a), without halo (type b), medium halo (type c) and big halo (type d). Counted sperms we were mentioned like as:

	Total Counted Sperm	Percentage (%)
Degraded (type – a)		
Without Halo (type – b)		
Medium Halo (type – c)		
Big Halo (type – d)		
No. of sperm evaluated		

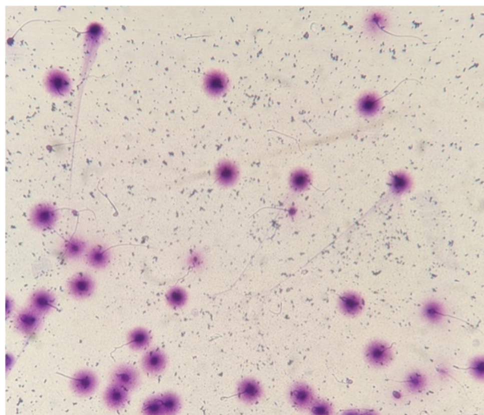
Lower Reference

$a + b \leq 30\ \%$

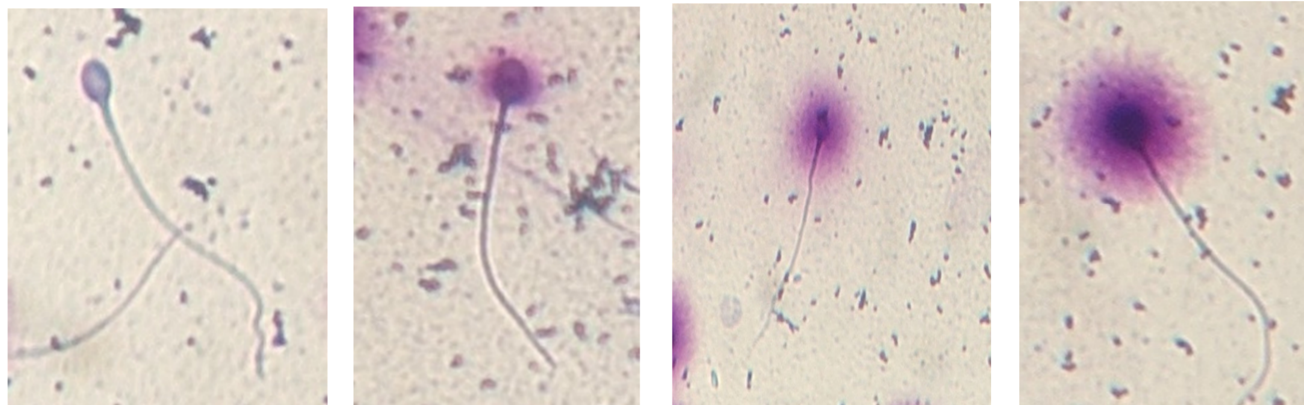
Total percentage of sperms with DNA Fragmentation (Type a +b): **%**

Total percentage of sperms without DNA Fragmentation (type c +d): **%**

Remarks:



Here is the original image of a slide showing the fragmentation of DNA in human sperm:



Degraded **Without Halo** **Medium Halo** **Big Halo**

3. **Data Collection:**

In this research we were collected the data from IRCC IVF Centre, Institute of Reproduction and Child Cares, Sector 17, Panchkula, Haryana. All patients were given informed patient consent for research purpose and permission from hospital authorities with maintaining confidentiality. Total 31 patients were enrolled for semen analysis but only 20 patients were selected for this study and collected data for dissertation writing and publication.

4. **Results of Patients Characteristics, SA data of Asthenozoospermia & Normozoospermia, Sperm DFI data of Asthenozoospermia & Normozoospermia**

Sperm DNA Fragmentation (SDF) was observed and it was found that the sperm DFI of Asthenozoospermia males were higher than the Normozoospermia males. According to the findings, sperm DFI was closely connected to infertility. despite being adversely correlated with increasing motility and sperm concentration. In this work, a generalised investigation of SDF between Asthenozoospermia and Normozoospermia parameters was assessed. Significant correlations between sperm DNA fragmentation and asthenozoospermic and normozoospermia semen samples were found. [23]. Results of patient's characteristic semen analysis, and DFI in Asthenozoospermia and Normozoospermia are mentioned in table 1.

Table 1: Patients Characteristics, SA data of Asthenozoospermia & Normozoospermia, Sperm DFI data of Asthenozoospermia & Normozoospermia

		Asthenozoospermia	**Normozoospermia**
Sr.	**Particular**	**Mean**	**Mean**
1	Male age	36.40 ± 5.60	31.26 ± 6.73
2	Female Age	32.80 ± 5.20	29.06 ± 6.93
3	Active marriage life	5.88 ± 10.12	3.90± 5.10
4	Miscarriage	0.2 ±0.8	0.33±0.66
5	Primary infertility	0.8±0.2 (70 %)	0.60± 0.40 (30 %)
6	Secondary infertility	005±095 (30 %)	0.25±0.75 (70 %)
7	Sexual Abstinence	4.20 ± 4.20	2.8 ± 2.20
8	Semen Volume	2.28 ± 2.26	3.08 ± 2.22
9	Semen pH	7.50 ± 0.10	7.26 ± 0.13
10	Sperm count	114.16 ±185.84	113.60 ± 96.40
11	Sperm Motility (a+b)	13.40 ± 11.60	53.06 ± 21.93
12	Leukocytes	0.55±2.24	0.46±1.84

13	Degraded (type a)	74 ± 123	15.60 ± 39.40
14	Without Halo (type b)	85.60± 88.40	25.93 ± 169.07
15	Medium Halo (type c)	234.60 ± 154.40	303.60 ± 138.40
16	Big Halo (type d)	105.80±79.20	154.86 ± 115.14

Our observational study found that the average age of Asthenozoospermic males was 36.40 years and Normozoospermic males was 31.26 years. Female average age was 32.80 years in Asthenozoospermic (AZS) and 29.06 years in Normozoospermic (NZS) couple. We noticed active marriage life was 5.88 years in AZS couple and 3.90 years in NZS couple. We were seen primary infertility on an average 0.8 years in AZS and 0.60 years in NZS couple. Primary infertility was found in AZS 70 % and 30 % in NZS couple. Secondary infertility was found in AZS 30 % and 70 % in NZS couple. We had recorded the patient's sexual abstinence on average 4.20 days in AZS males and 2.8 days in NZS males. After ejaculation and fluidification of semen sample we were measured the semen volume by using rubber free syringe and observed the average volume of semen was 2.28 ml in Asthenozoospermic males and 3.08 ml in Normozoospermic males. Semen pH is given to alkalinity of sample it was observed on an average 7.5 in AZS males and 7.26 in NZS males. Sperm count was seen 114.16 million / ml in AZS males and 113.60 million / ml in NZS males. Sperm motility [rapid progressive (type a) and slow progressive (type b)] on average was seen 13.40 % in Asthenozoospermic males and 53.60 % in Normozoospermic males. When leukocyte is higher, it is indicated urinary tract infection (UTI) and advised to the patient for semen culture. We observed leukocytes during this research and was found average 0.55 million / ml in AZS males and 0.46 million / ml in NZS males. During Sperm DNA fragmentation evaluation, we found degraded (type a) was 3.7 %, without halo (type b) 4.28 %, Medium halo (type c) 11.73% and Big halo (type d) 5.29 % in AZS males and degraded (type a) was 2.34 %, without halo (type b) 3.89 %, Medium halo (type c) 45.54 % and Big halo (type d) 23.23 % in NZS males.

5. Discussion:

The results demonstrated that sperm DF. Male infertility is significantly impacted by sperm DNA fragmentation, which also has a detrimental effect on the fertility of couples. In our investigation, we discovered that sperm DNA fragmentation was nearly strongly correlated with all sperm quality-related data, indicating that sperm DFI may be a major component in the deterioration of semen parameters (Hussein et al 2020). According to Aitken et al. (2010), the degree of sperm DNA integrity was associated with the quantity of reactive oxygen species (ROS) present in infertile men's seminal fluid. Examining infertile patients for ROS profiles and the sperm DNA integrity could be indicated for a better clinical management of infertile men. In the present study, the DNA fragmentation index (type-a) of fresh ejaculate was higher in asthenozoospermic patients and lower in normozoospermic patients. This is still of great concern, that increased SDF could reduce the full term pregnancy rate in ART (Zeke et al., 2012) and increase the risk of miscarriage (Liow et al., 2009), whereas generation of healthy offspring should be considered the goal of ART services (Thomson et al., 2010).

6. Conclusion:

One of the key factors causing male infertility, which is linked to poor reproductive results for couples, is the fragmentation of the sperm DNA. As a consequence of our findings, which indicated that sperm DFI may be a key factor contributing to the reduction of semen parameters, SDF was virtually strongly correlated with all values showing semen quality. [23].

In summary, we found a considerable higher SDF in asthenozoospermia sample and lower in normozoospermia sample and attribute it to presence of already existing higher SDF in their fresh ejaculates.

Funding:

No funding sources

Conflict of interest:

None declared

Ethical approval:

The study was approved by the Institutional Ethical Committee (IEC), Institute of Reproduction and Child Cares.

References:

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The international glossary on infertility and fertility care, 2017. Fertil Steril 2017;108:393-406.
2. Choy JT, Eisenberg ML. Male infertility as a window to health. Fertil Steril 2018;110:810-4.
3. Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertil Steril 2014; 102:1502-7.
4. Agarwal A, Allamaneni SS. Sperm DNA damage assessment: a test whose time has come. Fertil Steril 2005; 84:850-3.
5. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, et al.; National Cooperative Reproductive Medicine Network. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med 2001;345:1388-93.
6. Simon L, Emery BR, Carrell DT. Review: diagnosis and impact of sperm DNA alterations in assisted reproduction. Best Pract Res Clin Obstet Gynaecol 2017;44:38-56.
7. Künzle R, Mueller MD, Hänggi W, Birkhäuser MH, Drescher H, Bersinger NA. Semen quality of male smokers and nonsmokers in infertile couples. Fertil Steril 2003; 79:287-91.
8. Selevan SG, Borkovec L, Slott VL, Zudová Z, Rubes J, Evenson DP, et al. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect 2000; 108:887-94.
9. Calogero AE, La Vignera S, Condorelli RA, Perdichizzi A, Valenti D, Asero P, et al. Environmental car exhaust pollution damages human sperm chromatin and DNA. J Endocrinol Invest 2011; 34:e139-43.
10. Chatterjee R, Haines GA, Perera DM, Goldstone A, Morris ID. Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukaemia. Hum Reprod 2000;15:762-6.

11. Baskaran S, Cho CL, Agarwal A. Role of sperm DNA damage in male infertility assessment. In: Rizk B, Agarwal A, Sabanegh ES Jr, editors. Male infertility in reproductive medicine: diagnosis and management. Boca Raton: CRC Press; 2019;205.
12. Agarwal A, Prabakaran S, Allamaneni S. What an andrologist/urologist should know about free radicals and why. Urology 2006;67:2-8.
13. Esteves SC, Roque M, Bradley CK, Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. Fertil Steril 2017;108:456-67.e1.
14. Baskaran S, Agarwal A, Panner Selvam MK, Finelli R, Robert KA, Iovine C, et al. Tracking research trends and hotspots in sperm DNA fragmentation testing for the evaluation of male infertility: a scientometric analysis. Reprod Biol Endocrinol 2019;17:110.
15. Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, Zini A. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl Androl Urol 2016;5:935-50.
16. Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 2005;322:33-41.
17. Ashok Agarwal ,et al, Sperm DNA Fragmentation: A New Guideline for Clinicians ,Review Article pISSN: 2287-4208 / eISSN: 2287-4690 World J Mens Health 2020 Oct 38(4): 412-471 https://doi.org/10.5534/wjmh.200128 Male reproductive health and infertility
18. Mc Pherson S, Longo FJ. Chromatin structure-function alterations during mammalian spermatogenesis: DNA nicking and repair in elongating spermatids. Eur J Histochem 1993;37:109-28.
19. Rousseaux S, Boussouar F, Gaucher J, Reynoird N, Montellier E, Curtet S, et al. Molecular models for post-meiotic male genome reprogramming. Syst Biol Reprod Med 2011;57:50-3.
20. Shaman JA, Prisztoka R, Ward WS. Topoisomerase IIB and an extracellular nuclease interact to digest sperm DNA in an apoptotic-like manner. Biol Reprod 2006;75:741-8.
21. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, Irvine DS (1998) Rel- ative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod. 59:1037-1046.
22. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, Criminna R, Culasso F, Dondero F, Lenzi A, Spano M (2004) Full-term pregnancies achieved with ICSI de- spite high levels of sperm chromatin damage. Hum Reprod. 19:1409-1417. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, Criminna R, Culasso F, Dondero F, Lenzi A, Spano M (2004) Full-term pregnancies achieved with ICSI de- spite high levels of sperm chromatin damage. Hum Reprod. 19:1409-1417.

23. Hussein A. Fadhil , Ibtisam A. AL-Ali , Hameedah Hadi Abdul Wahid, Human sperm DNA fragmentation and its correlation with antioxidant markers and sperm characteristics in Iraqi males; EurAsian Journal of BioSciences Eurasia J Biosci 14, 4961-4968 (2020).