Original Article

Relationship between Sperm Parameters and DNA Fragmentation using a Halosperm Kit

Houshang Rafighdoost, Ph.D.[1], Mir Mehrdad Farsi, Ph.D.[2],
Maryam Javadi M.Sc.[1*], Soraya Khafri, Ph.D.[3,4]

1. Anatomy Department, Medicine School, Zahedan University of Medical Sciences, Zahedan, Iran
2. Anatomy Department, Medicine School, Babol University of Medical Sciences, Babol, Iran
3.Epidemiology and Reproductive Health Department, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Social Medicine and Health Department, Babol University of Medical Sciences, Babol, Iran

***Corresponding author,** E-mail address: maryamjavadi22@yahoo.com*

Received: February 2013 Accepted: April 2013

Maryam Javadi obtained her MSc of Anatomy from the Department of Anatomy and Cell Biology, Zahedan Univesity of Medical Sciences, Zahedan, Iran. Her research focuses on effects of sperm DNA fragmentation on semen parameters in infertile males by an improved SCD test, Halosperm

Abstract

Introduction: tegrity is the most important component for transferring genetic information Sperm DNA fragmentation is considered a component of male infertility. Its assessment comprises conventional semen analysis of which emphasis is placed on its evaluation in fertility clinics. In this study, we investigate the relationship between sperm parameters of motility, morphology, concentration, and DNA fragmentation level (DFLs) in sperm.

Materials and Methods: 89: nfertile men were assessed by conventional sperm analysis. DFLs were determined by Halosperm, a sperm chromatin dispersion test (SCD) which was performed in both groups of patients, the low DNA fragmentation group (LFG≤30%) and high DNA fragmentation group (HFG) 30%.

Results: Sperm parameters of concentration ($p<0.001$), motility ($p<0.001$) and morphology ($p<0.01$) in the HFG group were significantly lower than in the LF group after sperm preparation. Logistic regression model showed morphology and motility variables were predictive of DFLs. The cut off points were 5% (morphology) and 50% (motility).

Conclusion: Our results have shown a negative relationship between DFLs and sperm parameters after preparation. According to a DF value of >30% in the percent of men with normal semen parameters, the high importance of performing a DF test in the clinic setting in order to determine sperm DNA problems and the presence of sperm abnormalities in patients is recommended.

Keywords: Spermatozoa , Infertility, DNA Fragmentation

To cite this paper: Rafighdoost H, Farsi MM, Javadi M, Khafri S. Relationship between Sperm Parameters and DNA Fragmentation using a Halosperm Kit. Anat Sci J 2013; 10(2): 79-85.

Introduction

Assisted reproductive technology (ART) is composed of methods that aim for pregnancy and birth. Intra-uterine insemination (IUI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most common techniques in ART (1). There are many aspects for the success of ART, without doubt the quality of oocytes and sperm are the most important factors [2,3] Semen analysis is a routine test to evaluate sperm quality. Despite some pitfalls, the test result is generally acceptable and considered reliable in the assessment of male fecundity [4]. Nevertheless, semen analysis is subjective and sperm parameters vary based on patient conditions. However, they do not always reveal the quality and health of sperm [5]. Sperm DNA integrity is essential for accurate transmission of genetic information [3]. Apoptosis, reactive oxygen species (ROS) and abnormalities in chromatin packaging could be major sources for disintegrated sperm DNA [6,8]. It seems that genetic abnormalities in the paternal genome are one of the main causes for early pregnancy loss (EPL) [9].

To overcome this problem different methods have been proposed that might be more reliable and valuable than routine semen analysis. These methods evaluate sperm chromatin and DNA integrity related to male fecundity. The sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase-mediated deoxy-uridine triphosphate nick end-labeling assay (TUNEL), the single cell electrophoresis assay (COMET), and the sperm chromatin dispersion (SCD) test may reveal more defects in sperm competency [10-13]. These tests are to be correlated with sperm parameters in routine semen analysis [14]. The DNA fragmentation index (DFI) can be used as an independent predictor of fertility in couples undergoing ART [15]. Recently, an easy and fast diagnostic test based on sperm chromatin dispersion (SCD) [13], the Halosperm Kit has been developed. Sperm with fragmented DNA fail to produce the characteristic halo in SCD. Normal sperm without massive DNA fragmentation produce nucleoids with large halos of spreading DNA loops [16]. It is postulated that if the sperm DNA fragmentation (DF) value exceeds 30%, sperm quality decreases significantly [17]. Based on a DF quantitative value, it may be possible to choose the appropriate technique in infertility clinics. On the other hand, using the DF value for all patients is not cost-effective; therefore choosing patients based on their sperm parameters for the DF test is advisable. We investigated the prevalence of sperm DNA damage in a group of infertile men with both normal and abnormal semen parameters in order to elucidate whether Halosperm analysis can add to the information obtained by routine semen analysis in explaining the causes of infertility. In this regard, we have analyzed the relationship of sperm parameters with sperm DF by using the Halosperm Kit in patients who were candidates for ICSI.

Materials and Methods

This was a cross-sectional study performed on 89 infertile men whose wives were completely healthy. The Center's Ethical Committee approved the study. Patients underwent their first ICSI attempt because of male factor infertility or unsuccessful IVF and IUI procedures, and who referred to Mehregan Clinic and Fatemeh Zahra Fertility Center in Babol, Iran. After preparing semen samples by the swim up method, they were evaluated using a Halosperm Kit to determine the level of DF. Samples for ICSI were classified in two groups: i) patients whose sperm fragmentation level was >30% were considered to have high fragmenta-

tion (HFG) and ii) patients whose sperm fragmentation levels was ≤30% were considered to have low fragmentation (LFG) [16]. Participating patients gave consent to participate in the study.

Sperm analysis

Both the semen analysis according to World Health Organization guidelines (WHO, 1999) and DF assessment were performed on the day of oocyte pick-up. Specimens were collected with assistance of the female partner after 3-5 days of sexual abstinence and analysis were performed after liquefaction, by using a light microscope (Olympus/Japan). Sperm concentrations, morphology and motility were assessed before and after semen preparation. Sperm morphology was assessed according to Kruger's strict criteria after Papanicolaou staining. Sperm concentration was assessed by a Makler counting chamber (Bruckberg, Germany). Sperm motility was classified as either grades A, B or C and at least 100 spermatozoa were scored with a 40x objective. Total motility was calculated as the total of A and B motility rates.

Swim up

Semen samples were diluted at a 1:3 ratio with Ham's F10 medium without albumin (Sigma Aldrich, USA) and centrifuged for 10 min at 1500 rpm. After centrifugation, the supernatant was discarded. Next, 1 ml of Ham's F10 medium that contained 20% human albumin (Marburg, Germany) was layered on the pellet. The spermatozoa were allowed to migrate for 20 min at 37˚C and 5% CO_2. After 20 min, 0.5 ml of the supernatant were gently aspirated and placed in 5 ml conical tubes.

Assessment of sperm DNA fragmentation (DF)

Sperm DF was assessed after semen preparation using the Halosperm Kit (Parque Tecnológico de Madrid Spain). The semen samples were diluted to a concentration of 20 million sperm per ml. Then, spermatozoa were immersed in agarose microgel and spread on the slide. Samples were denatured with an acid and lysis solution, dehydrated and stained with Diffquick.

Sperm with large halos (thicknesses that were similar or larger than the length of the smallest diameter of the core) and sperm with medium-sized halos (thickness greater than 1/3 of the smallest diameter of the core and less than the smallest diameter of the core) were classified as 'spermatozoa having no fragmentation' (Figure 1).

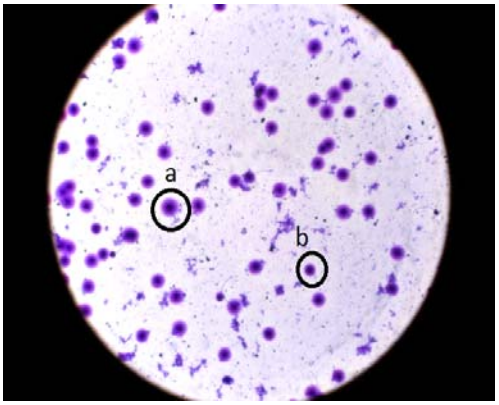

Figure 1. Image of patient's semen in LFG.
(a) sperm with large halo;
(b) sperm with medium-sized halo.

Spermatozoa with a small halo (thickness similar or smaller than 1/3 of the smallest diameter of the core) and those with no halo were classified as spermatozoa with DF (Figure 2).

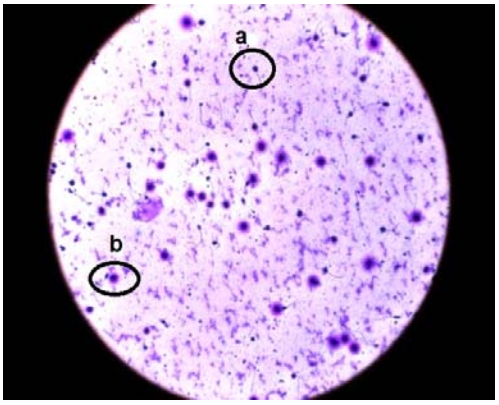

Figure 2. Image of patient's semen in HFG.
(a) Sperm with no halo; (b) sperm with small halo.

Statistical analysis

Statistical analysis was performed using SPSS software (version 18, SPSS Inc., Chicago, IL, USA). Categorical data were analyzed by the chi-square test and continuous data by the independent sample t-test. The cut off values were determined using ROC curve analysis. Sensitivity, specificity, positive and negative predictive values were calculated. A p-value less than 0.05% was considered statistically significant.

Results

In both groups, patients' ages were 5.5 ± 33.1) and, 5.5 ± 35.5, respectively. Patients were composed of (19%) 17 terato (17%) 15, astheno, and 4 (4.5%) oligozoospermic sperm. There were 25 (28%) who had more than one abnormal sperm parameter. Of 89 patients, 28(31.5%) were normal based on their semen parameters, however the IVF and IUI methods were not successful. They were chosen for the ICSI method. The results in Table 1 showed that in prepared sperm the average concentration, motility and normal morphology of sperm were significantly lower in HFG than LFG. (P<0.001, p<0.001 and p=0.008 respectively).

Table 1: Semen parameters after preparation in two group.

	HFG	LFG	P-value
Total motility rate (%)	49.53±3.82	76.60±2.38	<0.001
Normal morphology rate (%)	6.92±.71	12.58±.78	<0.001
Sperm concentration (million/ml)	21.91±4.11	36.41±3.42	<0.01

*Values are mean ± SE.

Multivariable logistic regression showed that only the morphology (p=0.01) and motility (p=0.002) variables were regarded as predictors. By taking these predictors into consideration, in relation to normal morphology the best cut off point was 5%, which had an accuracy of 0.72. At this cut off point, specificity was approximately 96%, which indicated that 96% of patients with DF ≤30% had a normal morphology of ≥5%. Test sensitivity was 53%. In other words, 53% of patients who had DF>30% had a normal morphology <5%. Negative predicted value (NPV) of the test was 75%, which indicated that 75% of patients with normal morphology of ≥5% had a DF of ≤30%. Positive predicted value (PPV) of the test was 90%, where 90% of patients whose normal morphology was <5% had a DF >30% (Table 2).

Table 2: Sensitivity, specificity and diagnostic accuracy of normal morphology for predicting DNA fragmentation (DF).

	DF ≤30	DF >30
Cycle started (n)	53	36
Normal morphology <5 (n)	2	19
Normal morphology ≥5 (n)	51	17

Sensitivity: 53% (36,69)
Specificity: 96% (91,100)
NPV:75% (65,85)
PPV: 90% (78,100)

In terms of motility, the best cut off point was 50%, which had an accuracy of 0.82. The specificity was approximately 98% and sensitivity was 36%. PPV of the test was 93% (Table 3).

With regards to the cut off points and the results of the multivariable logistic regression model, the OR for motility was 17.5 (1.91-161.67). OR for morphology was 20.5 (4.08-103.64).

Table 3: Sensitivity, specificity and diagnostic accuracy of motility for predicting DNA fragmentation (DF).

	DF ≤30	DF >30
Cycle started (n)	53	36
Motility <50 (n)	1	13
Motility ≥50 (n)	52	23

Sensitivity: 36% (20,52)
Specificity: 98% (94,100)
NPV: 69% (59,80) PPV: 93% (79,100)

Discussion

Most fertility clinics evaluate semen samples by conventional analysis, which does not ensure the absence of male infertility factors [18]. The results of our study show a negative relation between DFLs and the sperm parameters of motility, morphology and concentration following preparation.

In 2000, Irvin showed that infertile men who had weak sperm motility and abnormal morphology, had higher DNA fragmentation levels compared to those with normal semen samples [19]. Virro, by using the SCSA method researched DF on semen samples and showed that patients with DF, 30%< had one or more abnormal factors in their sperm. Even when semen parameters were normal, some had high levels of DF which had infertile factors hidden in them, and they were considered high risk for beginning pregnancy. Also emphasized was the necessity for assessment of this test for these men [17]. In 2008, Veles de la calla reported a negative relation between sperm parameters and sperm DF by the Halosperm assessment [20]. In 2010 Vilmaz used the halosperm assessment and reported that sperm motility in prepared samples in HFG was statistically lower than LFG [16]. On the other hand, some studies reported no relationship between sperm parameters and sperm DNA fragmentation [21, 22]. Reactive oxygen species (ROS) has been determined to be a very important factor in damage to sperm DNA. After this damage the cell membrane is dmaged and avoids sperm suitable motility [23, 24]

One of the notable points in our study was the determination of suitable cut off points for semen parameters following preparation. Our study showed that the best cut off point for motility related to DFL was 50%. Totally, according to the high PPV of the test, there was a high percentage of sperm DF in patients whose sperm motility was <50%. In the clinic, these patients had high potential DF, which shows the effect of high DF on decreasing motility. In accordance with the NPV of this test, approximately 69% of patients whose sperm motility was ≥50% had DF values of ≤30%. Among these, 31% had sperm motility of ≥50% and DF values of >30%. In the clinic, if this test was not performed, it was possible that their infertility factor would not have been recognized. Thus the DF test was necessary in these patients. The OR result was approximately 17.5, which showed a high effect of increased DF on decreased motility.

The current study showed the best cut off point for morphology that was related to DF to be 5%. Generally, according to a high PPV, the high percentage of sperm DF in patients whose normal sperm morphology was <5% was emphasized. In the clinic, these patients had high potential for DF, which shows the effect of high DF on decreasing normal morphology. According to the NPV of this test, approximately 75% of patients whose normal sperm morphology was ≥5% had DF values of ≤30%. Among these, 25% had normal sperm morphology of ≥5% and DF of >30%. In the clinic, if this DF test was not performed it was possible that their infertility factor would not be discovered. The OR of this test was about 20.5 which showed the high effect of increased DF on decreased normal

morphology. In 2008, Erenpreiss also reported that decreased normal sperm morphology and motility were important factors for predicting high damage of sperm DNA. Patients with motility <50% and abnormal morphology (normal morphology <5%) had higher OR for DF >30% than patients with normal sperm motility and morphology. A percentage of patients with normal semen parameters had high DF. The necessity of performing the DF test in these patients in order to reveal the infertility factor was emphasized [25]. In another study, with normal semen parameters, there were a number of patients with high levels of DF. Such factors were hidden, thus the possibility of a high risk for pregnancy existed [17].

Considering the results of the DF test, the possibility of using a suitable ART method existed if patients had a level of DF over 30%. ICSI would be more successful than IVF and IUI and should be considered as the method of choice [15].

In conclusion, our results showes that semen parameters after preparation had lower quality in patients with high DF. In addition, in some Patients with normal conventional semen parameters, DF was >30% that may show the necessity for assessment of the DF test in these cases. This test might be an appropriate tool for the evaluation of a patient's sperm prior to ART and might reduce the risks of using DNA-damaged sperm for fertilization. It might help to avoid any financial and emotional problems associated with failed ART.

Acknowledgement

This study was financially supported by Zahedan University of Medical Sciences. We would like to thank our IVF lab technicians.

References

1. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992; 340: 17-18.
2. Hull MG, Fleming CF, Hughes AO, McDermott A. The age-related decline in female fecundity: a quantitative controlled study of implanting capacity and survival of individual embryos after in vitro fertilization: Fertil Steril 1996; 65: 783-90:
3. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, et al. Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod 2004; 19: 1409-17.
4. Høst E, Ernst E, Lindenberg S, Smidt-Jensen S. Morphology of spermatozoa used in IVF and ICSI from oligozoospermic men. Reprod Biomed Online 2001; 3: 212-15.
5. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod 2003; 9: 331-45.
6. Kim JM, Ghosh SR, Weil AC, Zirkin BR. Caspase-3 and caspase-activated deoxyribonuclease are associated with testicular germ cell apoptosis resulting from reduced intratesticular testosterone. Endocrinology 2001; 142: 3809-16.
7. Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 2003; 79: 1597-605.
8. Manicardi GC, Tombacco A, Bizzaro D, Bianchi U, Bianchi PG, Sakkas D. DNA strand breaks in ejaculated human spermatozoa: comparison of susceptibility to the nick translation and terminal transferase assays. Histochem J 1998; 30 :33-9.
9. Li TC. Recurrent miscarriage: principles of management. Hum Reprod 1998; 13: 478-82.
10. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002; 23: 25-43.
11. Gorczyca W, Gong J, Darzynkiewicz Z. Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. Cancer Res 1993; 53: 945-51.
12. Hughes CM, Lewis SE, McKelvey-Martin VJ, Thompson W. comparison of baseline and induced

DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. Mol Hum Reprod 1996; 2: 613-9.

13. Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril 2005; 84: 833-42.

14. Spanò M, Bonde JP, Hjøllund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril 2000; 73: 43-50.

15. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. HumReprod 2007; 22: 174-9.

16. Yilmaz S, Demiroglu A, Yilmaz E, Sofuoglu K, Delikara N, Kutlu P. Effects of Sperm DNA Fragmentation on Semen Parameters and ICSI Outcome Determined by an Improved SCD Test, Halosperm. Int J Fertil Steril 2010; 4: 73-8.

17. Virro MR, Larson-Cook KL, Evenson DP. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 2004; 81: 1289-95.

18. Saleh RA, Agarwal A, Nelson DR, Nada EA, El-Tonsy MH, Alvarez JG, et al. Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study. Fertil Steril 2002; 78: 313-8.

19. Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ. DNA integrity in human spermatozoa: relationships with semen quality. J Androl 2000; 21: 33-44.

20. Velez de la Calle JF, Muller A, Walschaerts M, Clavere JL, Jimenez C, Wittemer C, et al. Sperm deoxyribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: results of a large prospective multicenter study. .Fertil Steril 2008; 90: 1792-9.

21. Khalili MA, Aghaie-Maybodi F, Anvari M, Talebi AR. Sperm nuclear DNA in ejaculates of fertile and infertile men: correlation with semen parameters. Urol J 2006; 3: 154-9.

22. Sills ES, Fryman JT, Perloe M, Michels KB, Tucker MJ. Chromatin fluorescence characteristics and standard semen analysis parameters: correlations observed in andrology testing among 136 males referred for infertility evaluation.J Obstet Gynaeco 2004; 24: 74-7.

23. Erenpreiss J, Spano M, Erenpreisa J, Bungum M, Giwercman A. Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J Androl 2006; 8: 11-29.

24. Wyrobek AJ, Eskenazi B, Young S, Arnheim N, Tiemann-Boege I, Jabs EW, et al. Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proc Natl Acad Sci USA 2006; 103:9601-6.

25. Erenpreiss J, Elzanaty S, Giwercman A. Sperm DNA damage in men from infertile couples. Asian J Androl 2008; 10: 786-90.