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Sperm DNA Fragmentation in Asian Men whose Partner had Repeated Miscarriage

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ABSTRACT

Introduction: ***Sperm DNA fragmentation (SDF) has been shown to adversely affect fecundity. We evaluate the level of SDF and semen analysis in men whose partners had miscarriages or successful pregnancy.***

Methodology: A prospective pilot study, collecting the ejaculates from men whose partner had two consecutive miscarriages (MG) was conducted from March to December 2008. These were compared to the control group (CG) where the ejaculates were collected from men whose partners were in their second trimester or recently given birth. The halosperm test was used to assess SDF. Semen analysis was performed according to WHO criteria. The level of SDF was measured as the primary outcome. Its correlation with semen analysis and lifestyle factors were analysed as secondary outcomes.

Results: We evaluated 35 and 23 semen samples from MG and CG respectively. We found a trend towards higher levels of DNA fragmentation in the MG (33.0vs28.9%CI-10.4-1.9,p=0.18) with no successful pregnancies observed where SDF>42%. SDF was inversely correlated with sperm motility (r=-0.46). Among lifestyle factors, smoker had higher SDF levels (33%vs30%,p=0.383) while frequent sauna visits was conversely associated with lower SDF (26vs33%,p =0.015).

Conclusion: SDF appears to affect pregnancy outcome. More studies are required to define the role of SDF in miscarriages.

Keywords: Halosperm, lifestyle factors, miscarriage, semen analysis, Sperm DNA fragmentation.

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INTRODUCTION

Excessive sperm DNA damage has been shown to affect fecundity.[1] Sperm chromatin integrity is essential for successful pregnancy and transmission of genetic material to the offspring. High sperm DNA fragmentation (SDF) has a high negative predictive value for the pregnancy outcome. Evenson et al. and Larson et al showed that DNA fragmentation Index (DFI) of >30% is incompatible with conception and normal pregnancy development.[2, 3] Lin et al reported that high DNA stainability of >15% is associated with spontaneous miscarriage in IVF, but not on ICSI.[4]

Greco et al had shown that antioxidant treatment with 1 g vitamin C and 1g vitamin E daily for 2 months could markedly reduce the percentage of DNA-fragmented spermatozoa.[5] Therefore, patients with high DNA fragmentation could be advised to delay expensive artifical reproductive treatment (ART) until improvement was shown on DNA fragmentation.

It is currently not known what the SDF levels are in the indigenous population in Singapore which has a unique mix of ethnicity, and the relationship between SDF and pregnancy outcomes. Here we investigated the level of SDF of a group of men whose wives are followed up in our hospital who either had a successful pregnancy, or had experienced recurrent miscarriages, and to correlate the SDF with semen analysis and lifestyle factors.

MATERIAL AND METHODS

Ethics

We conducted a prospective study, collecting the ejaculates from 2 cohorts of men whose partner were followed up in our hospital between March 2008 to December 2008. All participants gave written informed consent for this study and the protocol for the research was approved through the institutional research board (IRB), KK Women's and Children's Hospital.

Patient groups and sample collection

The miscarriage group (MG) involved men whose partners had two consecutive miscarriages and the ejaculates were collected within 3 months from the most recent miscarriage. We also collected the ejaculates from men whose partners were in their second trimester or gave birth to a healthy child within the recent 3 months as a control group (CG).

Patients with fertility problems, medical conditions such as diabetes, hypertenstion, peptic ulcer, renal failure, liver dysfunction, sexually transmitted diseases or testicular conditions such as unilateral or bilateral un-descended testes or varicoceles, and pregnancies resulting from assisted reproductive techniques (ART), were excluded from the study.

Semen samples were collected by masturbation at our fertility center or at home after a minimum abstinence of three days. For home collection, the semen samples were sent to laboratory for analysis within one hour.

The primary outcome was the percentage of SDF in both the MG and CG. The secondary outcomes include correlation of DNA fragmentation with standard semen analysis and lifestyle factors such as smoking, alcohol use, frequent laptop use (>1 hr/day) and frequent visits to sauna (>3 times a week).

Halosperm test kits (Halotech DNA SL, Spain) were used to assess DNA fragmentation (Figure 1). Semen analysis including pH, volume, concentration and motility were evaluated according to World Health Organization (WHO) criteria (1999). Morphology of sperm was evaluated following Tygerberg's strict criteria (Kruger et al., 1988). Both miscarriage and control groups were combined for analysis when secondary outcome measures were evaluated.

Statistical analysis was performed using PRISM 5 for windows. Descriptive analysis was performed for all variables listed in Table 1. Independent t-test with Welch's correction was used to compare DNA fragmentation between the two groups and also association of DNA fragmentation with lifestyle factors. A $p < 0.05$ was considered statistically significant. Pearson correlation coefficient was used to evaluate the correlation between DNA fragmentation and sperm parameters. A $r<0.3$ was considered as not having any significant correlation.

RESULTS

Following IRB approval, 35 semen samples from the MG and 23 semen samples from the CG were collected. Basic characteristics for the study population were illustrated in Table 1.

The MG had a higher levels of DNA fragmentation as compared to the CG (Table 2; Figure 2), although it did not reach statistical significance ($p=0.18$). Of note, there was no successful pregnancy where the DNA fragmentation level was above 42%.

Comparing both miscarriage and control groups, there were no statistical difference for use of laptop, frequent visit to sauna, smoking or alcohol use between both groups (see Table 3).

Figure 1:
Sperm DNA fragmentation determined using Halosperm test kit. Spermatozoa which are negative for DNA fragmentation develops either a large (A) or a small uniform halo (B) around the head. Spermatozoa which are mildly positive have very little halo around the head, while those which have significant DNA fragmentation does not develop any halo around the head (D). (A) and (B) were considered negative while (C) and (D) were considered positive for sperm DNA fragmentation in our study.

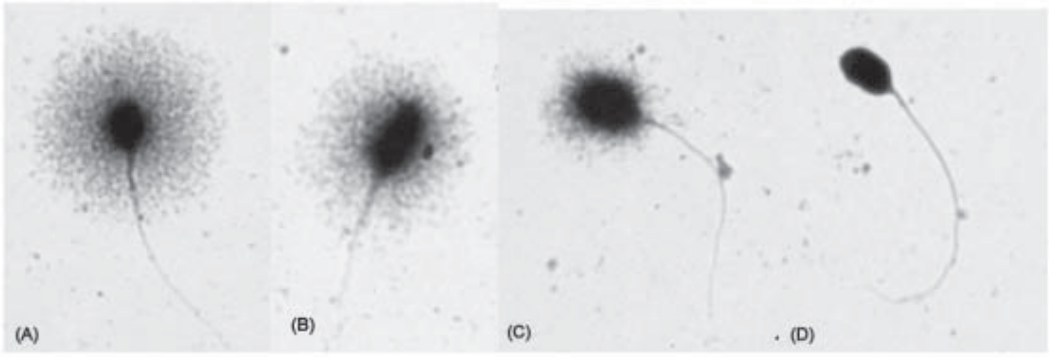

Table 1: Basic characteristics of study participants

Population characteristics	Miscarriage group	Control group
N	35	23
Age, median year (range)	36 (24-53)	32 (22-49)
Spousal (wife) age, median year (range)	35 (23-42)	28 (20-37)
Number of children, median (range)	0 (0-2)	1 (1-6)
Race		
Chinese	22	8
Indian	6	8
Malay	5	7
Others	2	
Years of marriage (mean)	5.1 (1-14)	4.1 (0.5-15)
Duration of unprotected sexual intercourse (median (range), months) before pregnancy	3 (0.5-24)	1 (1-12)
Previous contraception		
NIL	26	14
Condoms	8	10
COCP	0	1
IUCD	1	1
Contraception duration (mean, months)	13.6 (2-46)	5.9 (1-12)
Occupation		
Armed Forces	2	2
Banking	1	2
Clerical/Admin	2	1
Construction	3	1

Table 2: DNA fragmentation in men from the miscarriage group and the control group.

Item	Miscarriage group	Fertile group
Mean – SEM	33.1 – 2.57	28.9 – 1.8
Difference between means	4.2 – 3.1	
95% confidence interval	-10.4 to 2.0	
p value	0.18	

Fig. 2: DNA fragmentation in the miscarriage group and the control group.

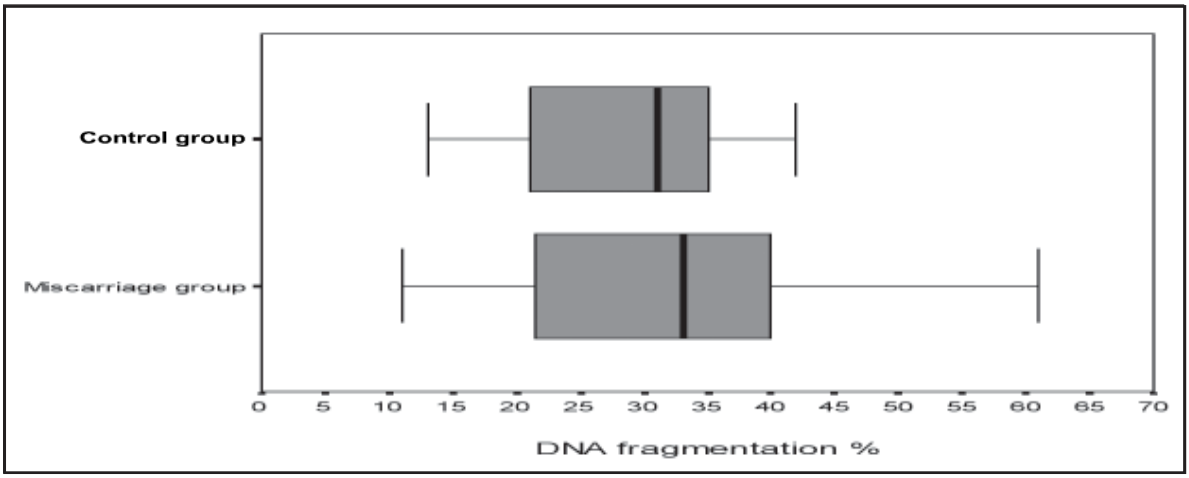

Table 3: Effect of lifestyle factors on sperm DNA fragmentation in the miscarriage group and the control group.

Item	Miscarriage group	Control group	P value
Frequent use of laptop (>1 hours a day)	1/35 (3%)	2/23 (9%)	0.335
Frequent visit to sauna (>3 times a week)	9/35 (26%)	8/23 (35%)	0.467
Smoking	16/35 (46%)	8/23(35%)	0.766
Number of cigarette (median)	12 (1-20)	10 (3-20)	
Alcohol use	1/35 (3%)	1/23 (4%)	0.897

Table 4: Correlation between sperm DNA fragmentation and lifestyle factors.

Item			DNA fragmentation % (mean +/- SEM)	P value
Frequent visit to sauna (>3 times a week)	Yes	17	26+/- 1.90	0.015**
	No	41	33+/-2.15	
Smoking	Yes	24	33+/-2.40	
	No	34	30+/-2.30	0.383
Smoking >15 cigarette	Yes	11	28.91 – 3.28	
	No	13	36+/-3.22	0.102
Frequent use of laptop (>1 hours a day)	Yes	3	32 +/-2.33	0.899
	No	55	31 +/-1.76	
Alcohol use	Yes	2	32+/-8.5	
	No	56	31+/-1.7	0.992

Fig. 3: DNA fragmentation and lifestyle factors. (A) Effect of frequency of sauna visit on sperm DNA fragmentation. (B) Effect of cigarette smoking on sperm DNA fragmentation.

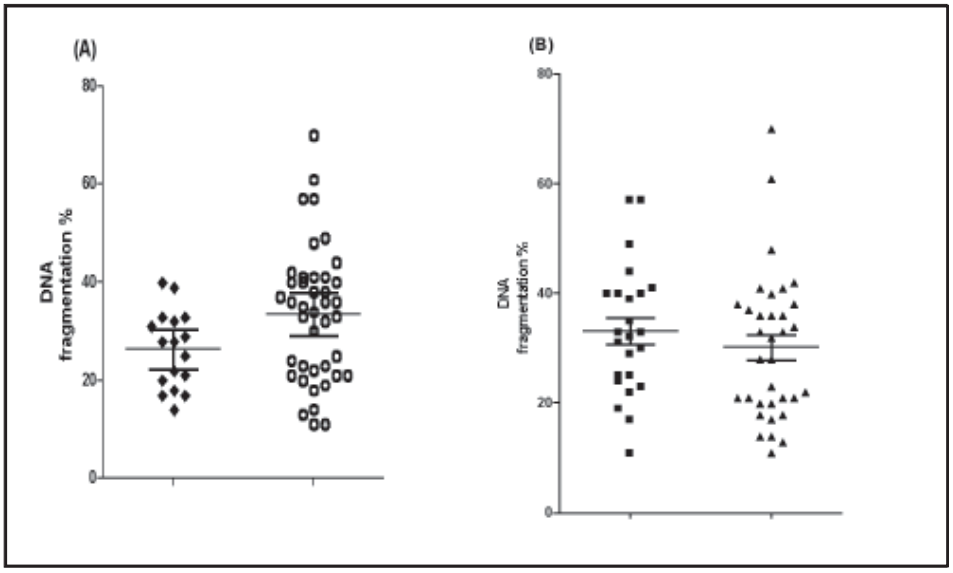

Fig. 4: Correlation of DNA fragmentation and sperm parameters, (a) motility, (b) morphology, (c) density and (d) volume.

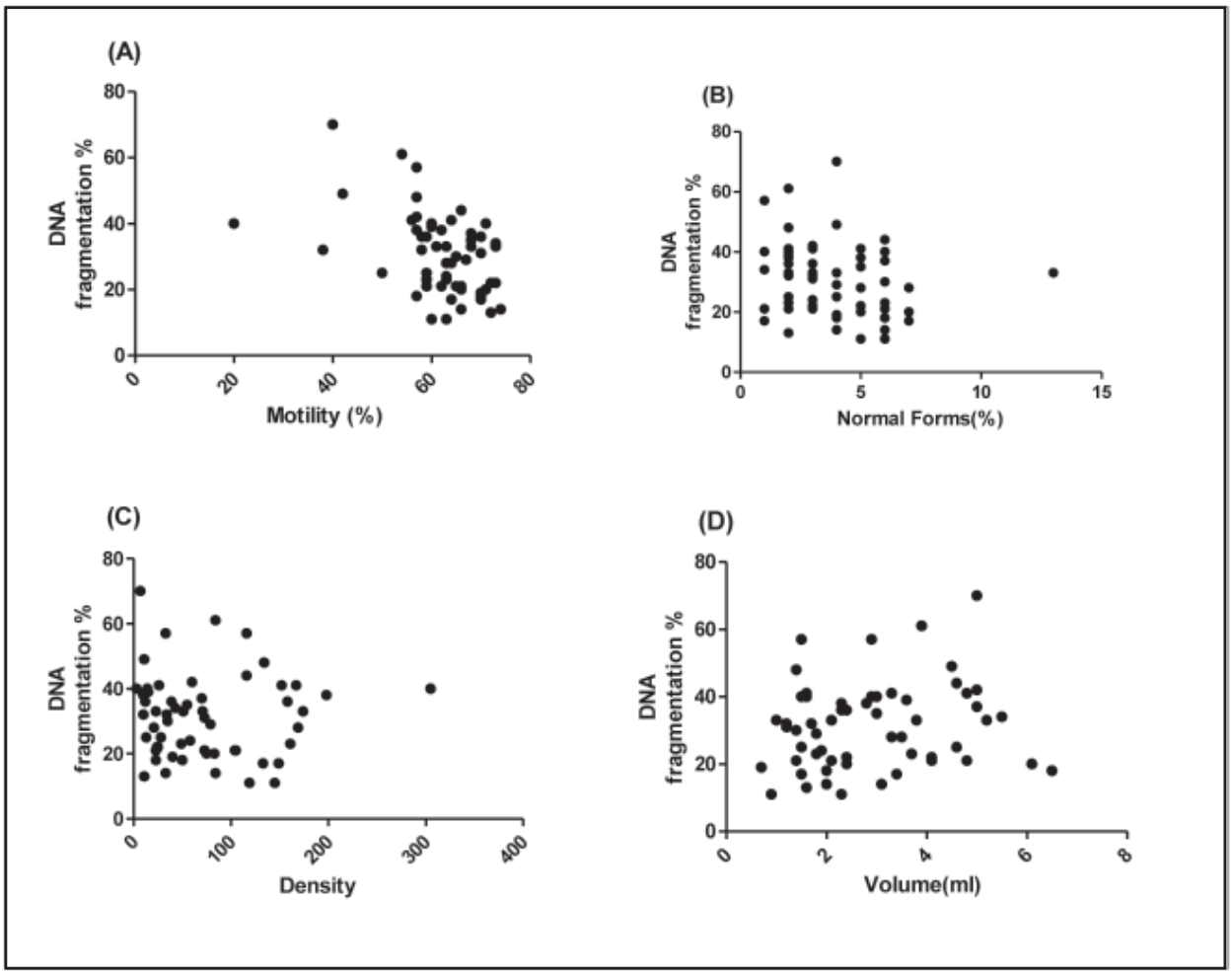

Table 5: Correlation between DNA fragmentation and semen parameters

Item	Pearson Correlation r	95% CI	P value
Volume	0.169	-0.09324 to 0.4096	0.204
Morphology	-0.249	-0.4790 to 0.01192	0.061
Concentration	-0.012	-0.2718 to 0.2494	0.929
Motility	-0.460**	-0.6437 to -0.2268	<0.001**

Among the lifestyle factors, smoking appears to be associated with higher SDF. However, there was no demonstrable dose-dependant relationship between the number of cigarettes smoked and level of SDF. We found that frequent sauna visits was associated with significantly less DNA fragmentation (p=0.015, table 4; Fig 3). There was no association between the frequent use of laptops and alcohol consumption with DNA fragmentation levels (Table 4).

The DNA fragmentation was found to be correlated negatively with sperm motility (r = -0.46, p <0.001) There were no statistical significance correlations between DNA fragmentation morphology, density or volume (Table 5 and Figure 4).

DISCUSSION

Male factor accounts for a significant proportion of subfertility cases seen at infertility clinics, with several authors having described a correlation between spermatozoa DNA damage with an adverse pregnancy outcome. Using a well validated DNA fragmentation index test, we present data which shows a wide variation of SDF in men who have either fathered successful pregnancies or fathered pregnancies which resulted in consecutive early miscarriages, to show that a SDF level above 42% was not compatible with a successful pregnancy. In addition, SDF levels was negatively correlated with spermatozoa motility.

Zini et al had performed a meta-analysis on seven studies and found that sperm DNA damage was significantly associated with pregnancy loss (OR 2.48; 95% CI 1.52-4.04; P <0.0001).[6] However, the author acknowledged that the predictive value may vary depending on the type of sperm DNA test used and cutoff level used. While most of the previous studies were conducted using Sperm Chromatin Structure Assay (SCSA), we used Halosperm in our study. Halosperm test kit is a simple test and provides a rapid result. It had also been proven to be cost effective and provide reliable and accurate results in assessing human SDF.[7]

Previous investigators have suggested that pregnancy is unlikely to occur when SDF is high. However, some investigators recently found that DFI level >30% was still compatible with pregnancy and delivery after either IVF or ICSI. They suggested that this could be due to the fertilization method of ART which helped overcome the impairment of sperm chromatin integrity. We found that DNA fragmentation level >30% was still compatible with spontaneous pregnancy and delivery without ART. However, there was no pregnancy noted with SDF >42%. Although there was no statistically significant difference between miscarriage group and fertile group, the mean DNA fragmentation was higher in miscarriage group.

Lifestyle factors play an important role in fecundity. Viloria et al and Spaniak et al found an increased rate of SDF in smokers.[8, 9] However, epidemiological data failed to demonstrate a significant reduction in fecundity. The waiting time to pregnancy is increased if number of cigarettes smoked per day is more than 15.[10] In our study, smokers had a non-significant higher mean percentage of DNA fragmentation. When the dose effect was considered, heavy smokers (>/= 15 cigarette a day) had a lower mean DNA fragmentation which suggest DNA fragmentation is not dose dependent for smoking.

Despite common belief, our study found that frequent sauna visits were associated with significantly lower DNA fragmentation. This result needs to be validated in studies with a larger sample size and controlled for confounding variables.

Literature has shown conflicting result on correlation between the traditional semen parameters such as sperm concentration, motility, morphology and DNA fragmentation. We found that DNA fragmentation was inversely correlated with sperm motility. Intact DNA may be a prerequisite for appropriate sperm motility. Although not statistically significant, we also observed a trend of negative correlation between sperm morphology and DF in our study (r = 0.23). Therefore, injecting morphologically normal sperm in ICSI may result in lower spontaneous pregnancy loss.

The main limitation in our study is the small sample size. Therefore the result may not be generalisable to the entire population. Our study was intended as a pilot study to gather information on the local population which will aid our counseling processes. This will be the first pilot study addressing the issue in Singapore.

To our knowledge, this is the first study on SDF and its effect on pregnancy outcomes in our local population. Assessment of SDF may have a role to play as simple lifestyle modifications and the use of anti-oxidants have been shown to improve SDF parameters. Further studies on this observation is now warranted, together with an intervention approach to improve on SDF levels and hence the pregnancy outcome.

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REFERENCES

1. *Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod. 2004; 19(3): 611-615.*

2. *Larson KL, De Jonge C, Barnes A, Jost L and Evenson DP. Relationship between assisted reproductive techniques outcome and status of chromatin integrity as measured by the sperm chromatin structure assay (SCSA). Hum Reprod 2000; 15: 1717-1722*

3. *Evenson DP, Jost LK, Marshall D et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999; 14,1039-1049*

4. *Lin MH, Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 2008; 90: 352–359.*

5. *Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the Incidence of Sperm DNA Fragmentation by Oral Antioxidant Treatment. J Androl 2005; 26(3): 349-353.*

6. *Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008; 23(12): 2663–2668.*

7. *Fernández JL, Muriel L, Goyanes V et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril 2005; 84(4): 842.*

8. *Viloria T, Garrido N, Hernandez JL, et al. Sperm selection by swim-up in terms of deoxyribonucleic acid fragmentation as measured by the sperm chromatin dispersion test is altered in heavy smokers. Fertil Steril 2007; 88: 523–525.*

9. *Sepaniak S, Forges T, Gerard H, et al. The influence of cigarette smoking on human sperm quality and DNA fragmentation. Toxicology 2006; 223: 54–60.*

10. *Hassan MAM, Killick SR. Negative lifestyle is associated with a significant reduction in fecundity. Fertil Steril 2004; 81: 384–392.*