

Correlation Between Sperm DNA Fragmentation and Conventional Semen Parameters among Different age Groups

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To study the relationship between conventional semen parameters and sperm chromatin condensation (DNA fragmentation index) using aniline blue-eosin staining method among patients of different age groups visiting the In-vitro fertilization (IVF) clinic. Retrospective study. Tertiary care infertility centre. A total of 240 patient semen samples were studied between the period of May 2015 to May 2016 for conventional semen parameters (WHO criteria) and DNA fragmentation index (DFI) using aniline blue- eosin staining method. Patients were separated into three groups: ≤ 30 years, 31-35 years and 36 years & above. Statistical analysis was performed using Pearson correlation co-efficient and regression tests on the groups. Sperm concentration (Millions /ml), motility(%), normal morphology(%), DFI (%). In each age group, i.e., ≤ 30 years, 31-35 years and 36 years & above, there was a significant and negative correlation between DFI and sperm concentration ($r = -0.50$, $r = -0.34$, $r = -0.49$ respectively; $P < 0.05$), motility ($r = -0.69$, $r = -0.66$, $r = -0.54$ respectively; $P < 0.05$) and normal morphology ($r = -0.86$, $r = -0.80$, $r = -0.75$ respectively; $P < 0.05$). Sperm DNA fragmentation index among the age groups was not statistically significantly ($P > 0.05$). Our study demonstrated that age is not a predictor of DFI. Whereas, sperm concentration, sperm motility and normal sperm morphology showed a significant association with DFI in all the age groups i.e., better the conventional semen parameters, lower the DFI.

Keywords: Aniline blue- eosin, DFI, sperm chromatin condensation, sperm concentration, sperm morphology, sperm motility.

Semen analysis has been widely used as an essential test for predicting male fertility, however, it cannot detect sperm chromatin abnormalities. Various studies have been performed in the past predicting sperm chromatin abnormalities as one of the trigger for infertility in men⁴. Sperm chromatin maturity is an essential factor for

spermatozoa's fertilization capacity and embryonic development¹⁻³.

Sperm chromatin abnormalities could occur at the time of spermiogenesis⁵, where specific nucleoproteins pack the nuclear DNA tightly into a highly condensed chromatin. Sperm chromatin condensation abnormalities result in nuclear

damage as DNA fragmentation/ denaturation that is associated with male infertility⁶. However, the exact mechanism(s) by which chromatin abnormalities or DNA fragmentation occur in human spermatozoa are not well understood.

Different methods have been used to evaluate sperm chromatin abnormalities or immaturity. Analysis of the sperm chromatin compaction is performed by aniline blue staining, which detects sperm chromatin immaturity⁷⁻⁸ and differentiates between histones and protamines. Blue stain is taken up by lysine rich immature nuclei of sperm cells, whereas, nuclei of sperm cells that are mature remain unstained⁸.

There has been a social trend of delaying family planning and parenthood among couples in today's world. Spermatogenesis continues throughout life, compared to oogenesis, where the decline in ovarian follicle numbers marks the end of females reproductive cycle⁹. Previous studies on Assisted Reproduction Technologies (ART) have shown that among couples seeking pregnancy, males were significantly elder in comparison to those who do not require ART (36.6 vs 33.5 years)¹⁰.

Studies on sperm quality, DNA damage and their correlation with ageing has brought a lot of interest because of an increase in paternal age in the recent years.

This study was conducted to analyse the correlation of conventional semen parameters and DNA fragmentation index (DFI) for sperm chromatin compaction using Aniline blue-Eosin staining among different age group patients visiting the ART clinic.

MATERIALS AND METHOD

Patient Selection

A total of 240 patient semen samples were studied between the period of May 2015 to May 2016 for conventional semen parameters (WHO criteria) and DNA fragmentation index(DFI) using aniline blue- eosin staining method. Patients selected for our study were less than 40 years old and were divided into three groups based on their age into ≤ 30 years, 31-35 years and 36 years & above. Patients with history of any pathogenic infection in the past 2 months, with OAT's,

Azoospermia, Necrozoospermia, Hematospermia, Pyospermia and smokers were ruled out. This study was carried at a Tertiary care infertility centre and permission to conduct the experiments was taken from the hospital ethical committee, also informed written consents were obtained from each patient.

Semen Analysis

Patients provide semen samples for analysis by masturbation after an abstinence of 3 to 6 days. After 30 minutes of liquefaction at room temperature, every sample was analyzed for the conventional semen parameters¹² and DFI using the Aniline blue- eosin staining method.

In this technique, sperms were stained with Aniline Blue- eosin as described in a previous report^{5,11,13}. 10 μ L of raw semen sample was smeared on a slide and subsequently air dried for each patient. Slides were fixed at room temperature in 4% formalin for 5 minutes, then rinsed with water and air dried. 5% aqueous aniline blue solution (HIMEDIA) (pH 3.5) was used to stain the slides for 5 minutes followed by rinse with water and air drying. Slides were then counter stained for 1 minute in 0.5% eosin (Merck), again rinsed and air dried.

Slide examinations were carried under bright field microscope at X 1000 magnification using oil immersion. Immature sperms stained dark blue, whereas the eosin counter stain, stained the mature sperms red pink. The percentage of abnormal sperm chromatin condensation or DNA fragmentation index (DFI) was recorded as the ratio of the number of dark-blue sperm to total number of sperm cells observed and multiplied by 100. A minimum of 200 sperm cells were observed for every slide.

Statistical Analysis

Statistical analysis was performed using Pearson correlation co-efficient and regression tests on the groups.

RESULTS

A total of 240 patient samples were studied and the average age of patients was 31.9 years. Patients were divided into three groups based on their age into ≤ 30 years, 31-35 years and > 36 years.

The percentage of sperm DNA fragmentation index(DFI) and semen parameters studied in the selected pool of patients showed a significant and negative correlation.

Table 1 shows that sperm concentration and DFI are negatively correlated to each other with a correlation co-efficient(r) of -0.50, -0.34 and -0.49 in the three groups.

In table 2 sperm motility and DFI between the different age groups shows significant and negative correlation with a correlation co-efficient(r) of -0.69, -0.66 and -0.54 in the three groups.

Table 3 also shows that sperm morphology and DFI are negatively correlated to each other with a correlation co-efficient(r) of -0.86, -0.80 and -0.75 in the three groups.

Also, our study demonstrated that sperm DNA fragmentation index between the different age groups was not statistically significantly ($P>0.05$). As shown in graph 1 where DFI among different age groups shows a linear pattern depicting that DFI is not related to age.

Table 1. Correlation of Sperm concentration and DFI among age groups

Age Groups	Sperm conc.(M/ml)	DFI(%)	Correlation co-efficient (r)	P-value
<= 30years (n=82)	71.5	16	-0.50	<0.05
31-35 years (n=128)	75	13.6	-0.34	<0.05
36 years & above (n=30)	78	15	-0.49	<0.05

Table 2. Correlation of sperm motility and DFI among age groups

Age Groups	Sperm motility(%)	DFI(%)	Correlation co-efficient (r)	P-value
<= 30years (n=82)	57.5	16	-0.69	<0.05
31-35 years (n=128)	58.5	13.6	-0.66	<0.05
36 years & above (n=30)	60.3	15	-0.54	<0.05

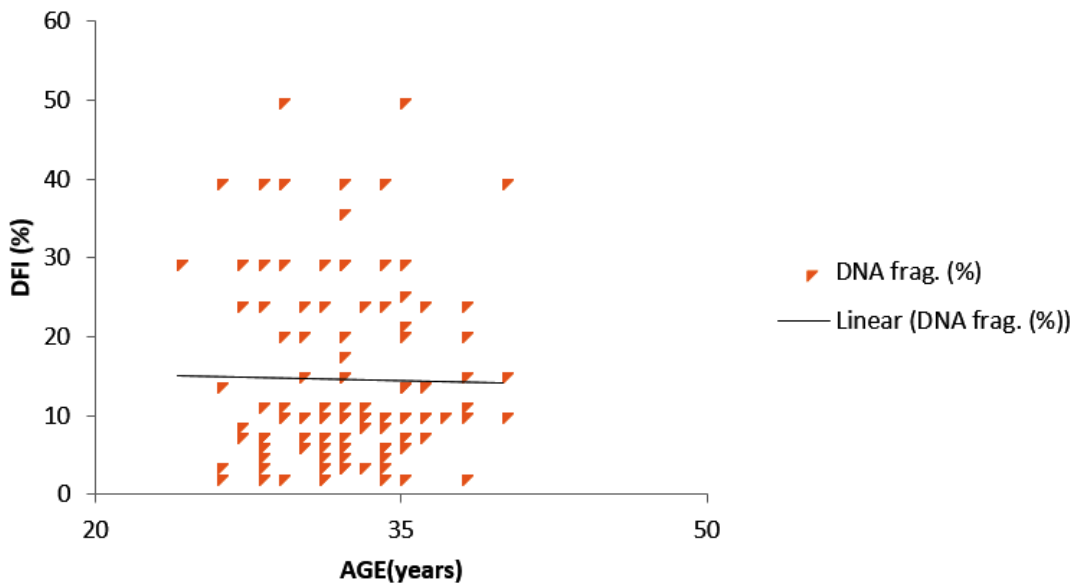
DISCUSSION

The results of our study demonstrated that some of DNA fragmentation index (DFI) has negative correlation to some of the semen parameters studied including sperm concentration, sperm motility and sperm morphology as shown in other studies^{13,20-22}, in contrast to studies that show positive correlation^{15-16,17-19}. Hammadeh *et al.*⁸ studied sperm chromatin condensation

using AB staining to assess male fertility and reported no correlation between sperm chromatin condensation and sperm count, motility and morphology. H.S.Kim *et al.*¹³ reported a negative correlation between abnormal sperm chromatin condensation and strict morphology. Dadoune *et al.*¹⁴ observed that normal morphology sperms had a higher percentage of normal sperm chromatin condensation heads than sperms with subnormal morphology.

Table 3. Correlation of sperm morphology and DFI among age groups

Age Groups	Normal morph.(%)	DFI(%)	Correlation co-efficient (r)	P-value
<= 30years (n=82)	12.7	16	-0.86	<0.05
31-35 years (n=128)	13.4	13.6	-0.80	<0.05
36 years & above (n=30)	13	15	-0.75	<0.05



Graph 1. Sperm DNA fragmentation index(%) vs Age (years)

Our study also shows that DNA fragmentation index (DFI) based on sperm chromatin compaction/condensation is an important factor to predict male fertility, along with the routinely studied conventional semen parameters^{8,13,22} among different age groups.

Sperm chromatin structure consists of a tightly packaged DNA and nuclear protein which are highly condensed, reducing the nuclear volume and head size¹⁶. Sperm chromatin condensation is an important process which involves the replacement of somatic histones by protamines during the phase of spermiogenesis¹⁷ which are responsible for the final condensation and the dense packaging of the sperm genome into unique tightly coiled “doughnut-loop” sub-units²³⁻²⁴. Sperm DNA degradation has been found to be associated with the presence of persistent histones as shown by various studies²⁵⁻²⁶ which affects male fertility. Therefore, it is important to evaluate sperm chromatin condensation during routine semen analysis in order to provide a better treatment option to patients with male factor and unexplained infertility.

Our study observed that DFI for sperm chromatin condensation had no relationship to ageing in men as shown in other studies²⁷⁻²⁸. A study by H.S. Kim *et al.*¹³ did not show any statistical significance of age of men on morphology and abnormal sperm chromatin condensation. Also, Nijs *et al.*²⁹ studied that there was no influence of age of men on sperm concentration, motility or morphology, also no significant increase in DNA fragmentation was observed in their study of 278 patients that underwent their first IVF or ICSI cycle. In contrast, studies have shown that men with advanced age are at higher risk of having children with chromosomal defects³⁰.

CONCLUSIONS

Our study demonstrated that age is not a predictor of DFI. Whereas, sperm concentration, sperm motility and normal sperm morphology showed a significant and negative correlation with DFI in all the age groups i.e., better the conventional semen parameters, lower the DFI. It's important to understand that DFI based on sperm chromatin compaction/condensation is a

very important parameter for the assessment of male fertility.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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