# DNA Content of Human Spermatozoa with Respect To Sperm Morphology

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## ABSTRACT:

**BACKGROUND:**

The studies on the relationships of sperm morphology and DNA was backdated for nearly 25 years. DNA and morphology was studied in mammalian sperm by flow cytometry and DNA content of different sperm types for normal and abnormal subfertile humans was recorded. The effects of some factors like drugs, cigarette smoking, cryopreservation and sexual abstinence and others on sperm morphology and DNA damage, denaturation and fragmentation in both normal fertile and abnormal infertile men were reported. Several methods for the evaluation and estimation of DNA in spermatozoa have been reported.

**AIM OF THE STUDY:**

The objective of the present study was the determination of the DNA content of spermatozoa in subfertile persons and its correlation with sperm morphology.

**METHODS:**

A random sample of 66 subfertile males attending the Institute for Embryo Research and Infertility treatment, University of Baghdad,were enrolled in this study through years 2004-2005.The patients age ranged 20-45 years and their semen samples were assessed for sperm morphology by direct light microscopy. DNA content (µg/ml) was estimated using a microchemical spectrophotometric method, and data were classified into two groups according to sperm percent abnormality (below and above 50%).

**RESULTS:**

The results showed that no statistically significant difference ($P > 0.05$) of DNA content between the two groups was obtained. The DNA content (µg/ml) showed a negative non significant correlation with percent morphology in the normal and abnormal groups ($P>0.05$).

**CONCLUSION:**

The level of the DNA content in the spermatozoa was not related to its percentage of morphological abnormalities .


***KEYWORDS:*** DNA, sperm morphology (percent abnormality), teratospermic .

## INTRODUCTION:

The sperm morphology (the size and shape) was one of the three sperm parameters (count, motility, and morphology) used to evaluate semen quality in both subfertile patients and normal fertile persons [1, 2, and 3]. It has been intensively dealt with throughout the years regarding factors that affect semen quality to various extents [4, 5] .Abnormalities in morphology (>50%) were termed teratospermia and had become common with the utilization of rigid criteria for the evaluation of sperm morphology. The studies on the relationships of sperm morphology and DNA was backdated for nearly 25 years .Flow cytometry of mammalian sperm (DNA and morphology measurement) was reported [6]. The DNA content of morphologically different sperm types for normal and subfertile humans was recorded [7, 8].

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Sperm morphology proved to be a more significant predictor of fertilization potential [9,10] and its impact on assisted reproduction [11]. The effects of cigarette smoking [12], cryopreservation of spermatozoa [13] and sexual abstinence period [14] on sperm morphology and oxidative DNA damage [5, 15] ,DNA denaturation (DD) [16,17, 18] and DNA fragmentation (DF) [12] in human sperm were reported. These were linked to sperm morphology in both normal fertile and abnormal subfertile men. Several methods for the evaluation and estimation of DNA in spermatozoa have been reported ([18, 19, 20, 21, 22, and 23]).

The present study dealt with the estimation of the level of DNA content of spermatozoa in a heterogenous group of subfertile men. The objective was to find a correlation between DNA content and the seminal sperm morphology (percent abnormality) in sub fertile men.

## MATERIALS AND METHODS:

The subjects were 66 subfertile males with different semen quality ranging in age from 20-45 years. Seminal fluid from each patient was obtained by masturbation after at least three days of sexual abstinence and examined microscopically within one hour of ejaculation. Liquefaction time ranges from 30-60 minutes. Ninty five percent of those men were primary subfertile type. Sperm morphology was observed in direct light microscope by placing a 10 µl drop of homogenized semen and using a micropipette onto a warm clean slide, and then covered with coverslip. Sperm morphological abnormalities (included head, neck and tail) was examined in this wet preparation. The abnormal sperm and total sperm counts were estimated from the mean of number of sperms in 10 random (40×) fields multiplied by factor of one million. At least 100 spermatozoa were counted. The following equation was used for calculating the abnormal sperm morphology:

$$\text{Percent abnormal sperm morphology} = \frac{\text{Number of abnormal Sperms}}{\text{Whole sperm count}} \times 100$$

The normal limit is (< 50%) according to WHO ([24]). Semen samples were deep frozen till the day of analysis. Frozen-thawed samples were used for the estimation of DNA content by the method based on the formation of color reaction of deoxyribose with indole [(19)] .An aqueous phase containing the stable colored indole-deoxyribose complex was read at 480 nm using Spectronic 20 spectrophotometer. The standard DNA solution (300µg/ml) was obtained from the Institute of Genetic Engineering, University of Baghdad. It was produced from human leukocytes in Tris-EDTA (TE) buffer pH 8.0 and was used to plot a standard curve. The blank solution consists of 1.0 ml of 0.3 M KOH, 0.5 ml 12M HCl and 0.5 ml 0.06 % aqueous indole solution and was processed exactly like the sample. Calculated samples for DNA content were expressed in (µg/ml) and categorized into two groups according to percent sperm morphology ( percent abnormality ) :

Group I ( teratospermic group , > 50 %) ; n=50 and Group II (normal group , < 50 % ) ; n=16 .

### STATISTICAL ANALYSIS:

Computerized statistical analysis was performed using SPSS (Statistical Package of Social Science), version 10.5 (Inc, Chicago, IL, USA) computer Software. Mean ±Standard Error of Mean of each parameter and Pearson`s correlation coefficient (r-value) between the two different parameters was done. The (P-value) of this was also calculated at (P< 0.05 ).ANOVA statistical analysis which is based on calculation of percentage points of F-distribution and LSD0.05 was applied to obtain the (P-value) between the two groups [(25)] .

## RESULTS:

Table 1, shows the values of DNA content (µg/ml) with respect to sperm percent morphology (% abnormality) represented as Mean ± SEM. The correlation coefficient (r value) between DNA content and sperm Concentration was also shown. Negative correlations (-0.09 and -0.17) were recorded in Gr.I and Gr.II, respectively. Those (r values were statistically non significant (P> 0.05) and could also be calculated from Figures (1 & 2).Table 2, shows the statistical Summary analysis of DNA content between the two groups. LSD0.05 measurement of Mean µg DNA/ ml between Gr.I vs. Gr.II was non significant (P>0.05).

**Table 1 : DNA content ( µg/ml ) vs. sperm percent morphology (% abnormality) in Group I (> 50 %) and Group II (< 50 % ) and its correlation (r value) with the percentage of abnormalities**

| Group | n | Sperm morphology (%abnormality ) | µgDNA/ml | r - value |
|---|---|---|---|---|
| Group I | 50 | 83.12 ±2.0 | 15.95 ±1.1** | - 0.09 ** |
| Group II | 16 | 45.60 ± 0.97 | 14.95± 0.97** | - 0.17 ** |

Values are Mean ± SEM

* * Means non significant (P>0.05)

**Table 2 : Statistical summary analysis of the data of dna content (µg/ml) between the two groups ,morphologically abnormal (group i) and normal (group ii) semen**

| Groups | n | Mean µgDNA/ml | LSD0.05 | P value |
|---|---|---|---|---|
| Group I | 50 | 15.95 | Gr. I vs. Gr. II=4.38* | > 0.05 |
| Group II | 16 | 14.95 | | |

* Means non significant (P> 0.05)

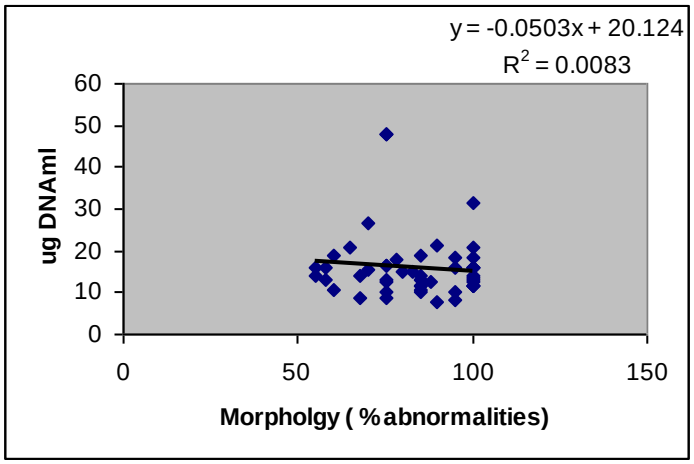


**Fig.1: Correlation of sperm morphology (percent abnormalities) With DNA content in morphologically abnormal semen (abnormalities> 50 % ).**

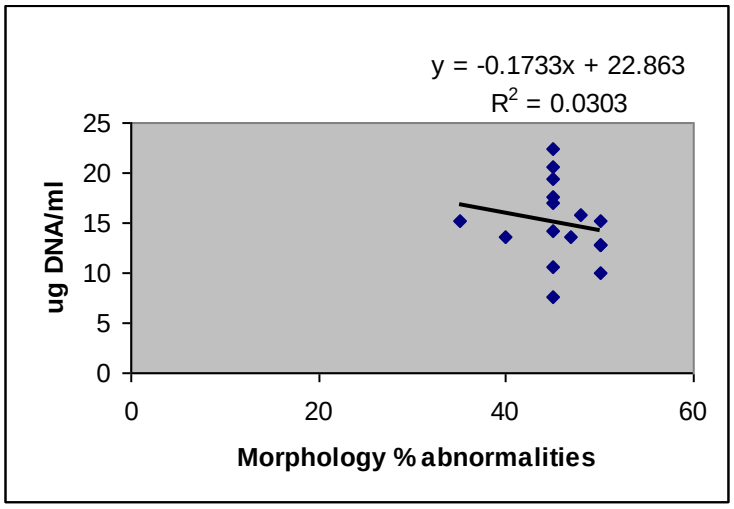


**Fig.2: Correlation of Sperm morphology (percent abnormality) with DNA content in morphologically normal semen (Abnormalities < 50 %).**

## DISCUSSION:

This study may reflect the necessity for determination of both the sperm DNA content and sperm morphology in assessing human sperm quality. This is in accordance with a previous report ([7]) .That work was on morphologically different sperm types of normal and subnormal males. It was reported that many heads with a strongly abnormal DNA content did not show any morphological abnormalities.

Those subcellular changes could be detected by the determination of the DNA content. It was also stated that patients with severe head abnormalities had lower chance of establishing successful pregnancies even though fertilization may be achieved [(10).] The presence of DNA strand breaks in both normal and oligospermic men was also reported ( [16] ) .The results presented in this study (Tables 1 & 2 ; Figures 1 & 2 ) revealed a negative correlation between sperm DNA content and sperm abnormal morphology. This finding was consistent with similar but more technical advanced studies made by several workers. Those workers reported that a negative correlation exist between the percentage of DNA fragmentation (DF) and morphology of the ejaculated sperm [(18, 22, 26)]. Other work was reported on oxidative DNA damage in human and association with morphology. Oxidative DNA damage was accomplished by assaying for 8-hydroxydeoxyguanosine (8-OHG), a precise and sensitive biomarker of oxidative stress, in both infertile and fertile. Moreover, studies on the two markers of sperm integrity, the DD and DF in fertile and infertile men indicated a negative correlation with morphology ([17, 27]).

## CONCLUSION:

The level of the DNA content in the spermatozoa was not related to its percentage of morphological abnormalities. More technically advanced work would be required in order to conclude the impact of sperm DNA integrity and concentration on its morphology and other semen quality parameters.

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