DNA content of Spermatozoa with respect to Seminal Sperm Concentration using a Microchemical Spectrophotometric Method

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Summary:

Background: *To determine the DNA content in subfertile patients and to correlate it with seminal sperm concentration.*
Design: *Prospective observational study.*
Setting: *College of Medicine, Dept. of Physiological chemistry and Institute for embryo Research and infertility treatment-University of Baghdad.The study was conducted through years 2004-2005.*
Methods: *A random sample of 61 subfertile male patients undergoing semen evaluation and aged from 20-45 years were studied. Semen samples were assessed for seminal sperm concentration microscopically and were classified into 3 different groups according to count (million/ml).Then sperm D N A content (µg/ml) was estimated using a microchemical spectrophotometric method.*
Results: *The three groups were statistically of significant difference(P <0.01).The D N A concentration per spermatozoan in Gr.III (1 20 million/ml) was higher than the other two groups It was also noticed that there was a statistically significant (P< 0.05) correlation in Gr.III b etween D N A content and sperm concentration . No significant (P >0.05)correlation was observed in the other two groups (Gr. I & Gr. II).*
Conclusion: *Significant positive correlation was obtained between sperm concentration and DNA content. in the oligozoospermic subfertile patient , but no significant correlation was found in the normozoopermic patients . D N A content per spermatozoan from oligozoospermic patients was higher than that in normozoospermic patients.*
Keywords : *DNA, Sperm concentration (count million/ml),* ***Normozoospermic, Oligozoospermic.***

Introduction:

The deoxyribonucleic acid (DNA) of spermatozoa from human with reduced fertility has been investigated with different methods. These are :colorimetric (1,2,3) fluorimetric (4,5) cytometric using flow cytometer (6,7) electrophoretic (comet) assay for DNA damage (8,9) and TUNEL method for DNA fragmentation(10).

The research studies on the DNA content of spermatozoa are often contradictory favoring in turn a lower, equal or higher DNA values in infertile men (3, 11, 12).

The present study deals with the evaluation of the level of DNA content of spermatozoa in subfertile groups of patients. A microchemical colorimetric method of Frajese et al (3) was applied. The objective of this study was to find a correlation between DNA level and the seminal sperm concentration(count million/ml) in subfertile patient groups.

Materials & Methods:

The subjects were subfertile male patients 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 macroscopically & microscopically within one hour of ejaculation. Liquefactions time ranges from 30-60 minutes. 95 % of patients were of primary subfertile type.

The concentration of the sperm count per mililiter was estimated from the mean number of sperms in 10 random (40 X) fields multiplying the mean number by a factor of one million. The normal lower limit is (> 20 million/ml) according to WHO (13).

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 was established first by Ceriotti (1) and modified latter by Frajese et al (3). An aqueous phase containing the stable colored indole-deoxyribose complex was read at 480 nm using Bausch & Lomb (Spectronic 20) Spectrophotometer. The standard DNA solution (300µg/ml) was obtained from Institute of Genetic Engineering - University of Baghdad It was prepared from human leukocytes in Tris-EDTA (TE) buffer pH 8 and was used to plot a standard curve.

The blank solution consists of 1.0 ml 0.3 M KOH, 0.5m1 12M HCL and 0.5m1 0.06% aqueous indole solution and was processed exactly like the sample.

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Calculated DNA were expressed in (pg/ml) and categorized into three groups according to sperm concentration (count million /ml) as follows:

Group I (>40 million/ml)	n=21
Group II (20-40 million/ml)	n= 9
Group III (1-20 million/ml)	n=31

Statistical Analysis

Computerized statistical analysis were performed using SPSS (statistical package of social sciences), version 10.5 (Inc., Chicago, IL USA) computer software. Meant Standard Error of Mean of each parameter and Pearson's correlation coefficient (r values) between the two different parameters was done. The (P values) of these(r's)were also calculated at (P<0.05)
ANOVA statistical analysis which is based on calculation of percentage points of F-distribution and LSDO.05 was applied to obtain the (P values) among group of patients (14).

Results

Table 1, shows the values of DNA content (pg/ml) with respect to sperm count (million/ml) represented as Mean ± SEM. The statistical mean values among the three group of patients were of significant difference (P<0.01). The DNA content per spermatozoan is increased as the sperm concentration is decreased.

The correlation coefficients (r values) between DNA content and sperm concentration are also shown in Table 1. A positive direct relation (values +0.33 and +0.61) were recorded for Group I and Group III respectively. However, an inverse relation (- 0.41) value was recorded for Gr.II . The (r values) were calculated from Figures 1,2,&3 for Gr.1,Gr.II, & Gr.III respectively and was statistically insignificant in Gr. I & Gr. II (P > 0.05) but was significant in Gr.III (P < 0.05) .

Table 2 , shows the statistical summary analysis of DNA content between the Groups of patients. LSDo.o5 measurement of Mean µg DNA/ml between Gr. I vs. Gr.III showed a non significant difference (P>0.05). Other LSDO.05 groups value, Showed a statistically significant difference between Gr.I vs Gr.II and Gr.II vs. Gr.III.(P<0.05).

Discussion

The values of our results appeared remarkably homogenous in the different groups and therefore, confirmed the reliability of the method for spermatozoal DNA determination.
The DNA content in spermatozoa from subfertile oligospermic group (Gr.III)was higher(4.8µg DNA/spermatozoan) than the two normozoospermic groups(Gr.I & Gr.II).This finding is in agreement with that of Oforofuo et al(15) and also compatible with that of Parez et al(16). However it was contradictory with that observation reported by Leuchtenberger et al (11) using a microcolorimetric method. They found wide variations in the DNA content of spermatozoa from infertile patients compared with that of normal men and a lower DNA content in the patients with oligospermia. Their patients appeared to form a rather poorly hetrogenous group. Although, our subfertile patients were also of hetrogenous group, the results obtained were in full agreement with those of Frajese et al (3) working on oligospermic patients affected by idiopathic spermatic arrest. It can be stated here that the spermatozoa of our infertile oligozoospermic patients had a DNA content several times higher than that of normozoospermic patients. This finding may be explained tentatively on the bases of genetic and / or cytologic factors such as a lack of reduction during meiosis or doubling spermatozoa, as has been reported by Fechneimer (17). ,

The weak non significant (P>0.05) correlation between sperm concentration and DNA content in Gr.I & Gr. II (two normozoospermic groups) was in agreement with that found by Oforofuo et al (15) It was reported that no significant quantitative correlation was found between sperm density among normospermic male patients. However,the significant correlation in Gr. III (oligozoospermic group) was contradictory with that report.

Table 2 , indicates, that there was a statistically significant differences (P<0.05) between Gr.II compared to Gr.I & Gr. III This may indicate that sperm concentration at this level in this normozoospermic Gr. II (20-40 million/ml) may contribute to the DNA content in the semen sample. However, such explanation may not be true since sperm concentration above and below this range in other normozoospermic Gr.I (>40) and. in the oligozoospermic Gr.III (1-20) showed no statistical difference in their DNA content (P>0.05).

Acknowlegment

We are indebted to Dr. Salwa Al- Awady in the Institute of Genetic Engineering , University of Baghdad for supplying the standard DNA solution.

Table 1 : DNA content (µg/ml) vs. sperm Groups; Gr.I (>40) Gr.II (20-40) and Gr.III (1-20) and their correlation coefficients (r values).

Group		Sperm conc.	µg DNA/ml	µg DNA/sperm	r-value
Gr	21	64.4 ± 3.3**	14.8 ± 0.81	0.24 ± 0.06	+0.33
Gr	9	32.9 ±2.3**	17.8 ±2.40	0.58 ± 0.11	-0.41
Gr	31	08.1 ± 1.4**	14.3 ± 0.65	4.8 ± 0.95	+ 0.61*

*Values are Mean ± SEM * Means Significant P < 0.05 ** Means Significant P < 0.01*

Table 2 : Statistical Summary analysis of the data of DNA content µg/ml between the three Groups of patients

Groups	n	Mean µg DNA/ml	LSDO.05	P-value
Gr. I	29	14.8	Gr.I vs. Gr. II = 2.2*	< 0.05
Gr. II	9	17.8	Gr. I vs. Gr.III =1.58	> 0.05
Gr. III	31	14.3	Gr.II vs.Gr.III = 2.1*	< 0.05

* Means Significant P < 0.05

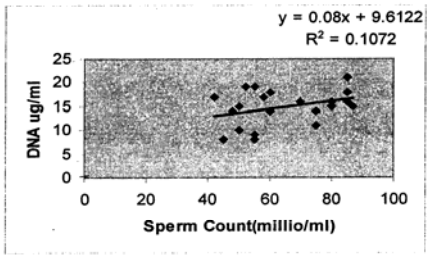

Fig.1 : Correlation coefficient of the data of sperm DNA content (µg /ml) vs. sperm count (million/ ml) of Gr.I of patients (>40).

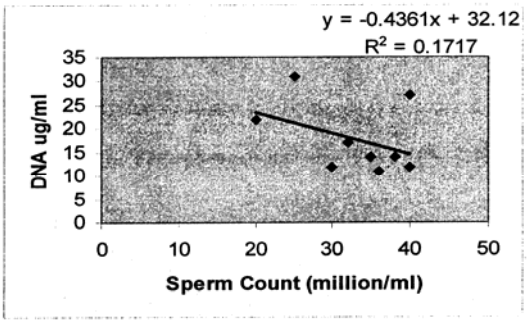

Fig. 2: Correlation coefficient of the data of sperm DNA content (µg/ml) vs. sperm concentration count (million/ml) of Gr. II of patients (20-40).

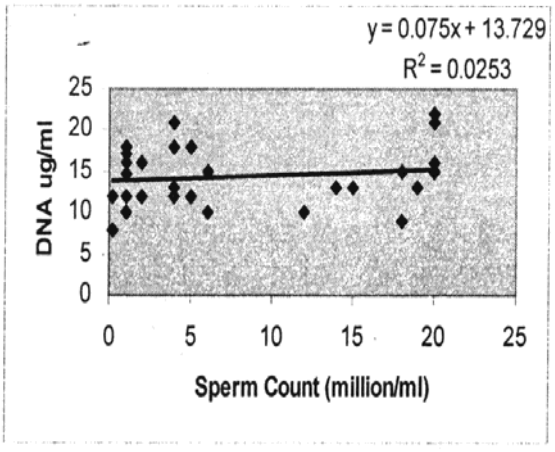

Fig.3: Correlation coefficient of the data of sperm DNA content (µg/ml) vs. sperm count (million/ml) of Group III of patients (1-20)

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