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Comparative analysis of semen quality parameters and DNA fragmentation in fertile and infertile males

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Abstract

This study aimed to assess the relationship between sperm DNA fragmentation and semen quality indicators in infertile males. 30 semen samples from fertile controls and 60 from infertile males with aberrant semen parameters made up the 90 semen samples examined. The semen was analyzed using the WHO (2010) standards, which include leukocyte count, morphology, motility, and sperm concentration. DNA damage was evaluated using the aniline blue staining method, and samples were categorized based on the extent of fragmentation.

All analyzed factors show statistically significant differences (P<0.001), according to the results. The normal group's mean sperm concentration was significantly greater at 54.93±13.44 million/ml than the abnormal group's, which was 17.10±15.24 million/ml. Similarly, the aberrant semen group's progressive motility was significantly lower (19.53±15.30%) than that of the normal group (56.43±16.85%). The normal group's sperm morphology (61.67±13.08%) was superior to that of the abnormal group (36.33±14.25%). Additionally, the normal group's semen volume (3.85±1.18 ml) was substantially greater than that of the abnormal group (3.26±1.47 ml). Furthermore, the abnormal semen group's leukocyte concentration (1.92±1.21 million/ml) was substantially higher than that of the normal group (0.68±0.28 million/ml).

According to the results, abnormal samples had significantly more DNA damage (49.50 ± 27.50) than normal samples (19.10 ± 5.31). Sperm DNA damage was much higher in the abnormal group than in the control group (P < 0.001). There was a statistically significant negative correlation between DNA fragmentation and sperm motility (r = -0.47, P = 0.001), morphology (r = -0.44, P = 0.001), and concentration (r = -0.41, P = 0.001). There was also a somewhat positive correlation between DNA fragmentation and leukocytes (r = 0.21, P = 0.052). This study found that infertile guys' low semen quality is closely correlated with greater sperm DNA fragmentation, which might be a good indicator for male infertility diagnosis.

Keywords: Male infertility, leukocytes, sperm motility, sperm DNA fragmentation, semen analysis.

Introduction

After a year of unbroken marriage and without the use of birth control, it is called infertility [1]. It is established that sperm DNA quality is necessary in maintaining the reproductive potential of men [2]. The fertilizing potential of sperm depends not only on the functional competence of spermatozoa but also on sperm DNA integrity [3]. There are two types of infertility: are primary and secondary. The women who have never experienced a clinical pregnancy are considered to have primary infertility [4]. Men have become infertile if their sperm characteristics are within the WHO normal range [5]. Several studies have investigated the relationship between sperm DNA fragmentation and semen parameters, like concentration, motility, and morphology [6]. SDF can occur at the cellular level due to apoptosis, increased oxidative stress, and abnormal chromatin condensation. These defects decrease fertilization potential, embryo quality, and overall reproductive outcomes. Reduced odds of conceiving naturally and decreased success rates in assisted reproductive technologies such as ICSI and IVF have been linked to increased levels of sperm DNA damage. Therefore, combining SDF testing with conventional semen analysis may enhance the diagnostic and prognostic evaluation of male infertility [7]. Sperm DNA integrity has an important role not only in fertilization but also in fetal development and normal embryo [8]. Cigarette smoking, medications, sperm leukemia, radiation, and varicocele are among the causes that lead to Increased levels of DNA breakdown in sperm and thus affect male fertility [9]. Research suggests that infertile males tend to generate sperm with greater DNA fragmentation, which may negatively affect the embryo's early development and pregnancy, like a higher risk of disorders in the progeny, such as childhood malignancies. A decrease in capacity for natural fertility is associated with a greater proportion of spermatozoa that have DNA damage [10]. This study aims to determine the correlation of sperm DNA damage with semen parameters in fertile and infertile men.

Materials and methods

Experimental design

This study was conducted at the Fertility Center of Al-Sadr Medical. City/Najaf, Iraq, and a Private infertility clinic in Najaf province. The research period spanned from November 2024 to April 2025. Semen samples were taken from fertility center patients who had abnormal semen, in addition to the control group that attended the fertility center. The age range for infertile males was between 21- 55 years, while fertile males ranged from 25 to 50 years. Perform semen analysis according to WHO (2010) criteria to classify samples as normal or abnormal based on semen parameters, including sperm concentration, motility, and morphology. Calculate the DNA fragmentation index (DFI) using the aniline blue staining method. These results indicate that increased DNA fragmentation reflects compromised spermatogenesis and semen quality parameters, contributing to male infertility.

Collection samples

Semen samples were collected from infertile males and controls after 3-5 days of sexual abstinence

directly in a dry, clean, and sterile disposable container by masturbation in a quiet room adjacent to the laboratory of seminal fluid tests. The container was marked with the information, including the patient's name and the age of the sample collection. The collected specimens were incubated at 37ºC for 30 minutes in an incubator to allow liquefaction. The liquefied specimens were mixed carefully for a few seconds and then examined under a microscope.

The analysis and classification of infertile men were performed according to WHO (2010), which was utilized to estimate the results of the seminal fluid.

Statistical analysis

The results were subjected to statistical analysis and analyzed using three statistical software, Microsoft Excel 2013, SPSS-27 (statistical package for social science, version 27), and MedCalc software (version 14.25).

The findings were presented as mean±SD (standard deviation) and numerical values. The Independent Sample t-test is used to calculate differences between two variables. To compare more than two variables, One-Way ANOVA (Analysis of variance) and Fisher's Least Significant Difference (LSD) were applied. The correlation of parameters was determined using Pearson's correlation coefficient. A *P*-value of less than 0.05 was considered statistically significant [11].

Results

Table (1) Comparison of Semen Parameters Between Normal and Abnormal Semen Groups

semen parameter	normal semen, n=30	abnormal semen, n=60	P value
Sperm concentration(million/ml)	54.93±13.44	17.10±15.24	<0.001*
Progressive motility (%)	56.43±16.85	19.53±15.30	<0.001*
Normal sperm morphology (%)	61.67±13.08	36.33±14.25	<0.001*
Semen volume(ml)	3.85±1.18	3.26±1.47	<0.001*
leukocytes(million/ml)	0.68±0.28	1.92±1.21	<0.001*

Data represented as Mean(±SD), *= significant at P≤0.05

Table (1) Compares the semen parameters of people with normal semen profiles (n = 30) with those of people with aberrant semen profiles (n = 60). All analyzed factors show statistically significant differences (P<0.001), according to the results. The normal group's mean sperm concentration was significantly greater at 54.93±13.44 million/ml than the abnormal group's, which was 17.10±15.24 million/ml. Likewise, the aberrant semen group's progressive motility was significantly lower (19.53±15.30%) than that of the normal group (56.43±16.85%). The normal group had significantly superior sperm morphology (61.67±13.08%) than the abnormal group (36.33±14.25%). Additionally, the normal group's semen volume (3.85±1.18 ml) was substantially greater than that of the abnormal group (3.26±1.47 ml). Additionally, the concentration of leukocytes was significantly elevated in the abnormal semen group (1.92±1.21 million/ml) relative to the normal group (0.68±0.28 million/ml).

Table (2) Comparison of DNA in the abnormal semen and normal semen groups

Parameter	normal semen, n=30	abnormal semen, n=60	P value
DNA (%)	19.10±5.31	49.50±27.50	<0.001*

Table (2) compares the level of DNA damage in normal and abnormal semen samples statistically. The results indicated a considerable deterioration of sperm DNA integrity in abnormal instances, with abnormal semen showing a much higher amount of DNA damage (49.50 ± 27.50) than normal samples (19.10 ± 5.31).

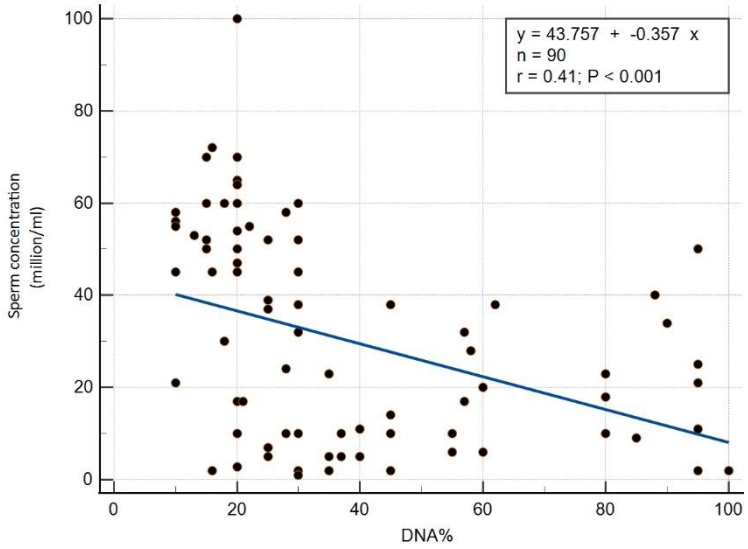

Figure (1) The correlation between DNA integrity of seminal plasma and sperm concentration

Figure 1 Shows how the quantity of sperm (measured in millions/mL) and DNA integrity (represented as DNA fragmentation percentage, or DNA%) relate to each other in seminal plasma samples. These two factors have a statistically significant negative association. The regression equation is expressed as (y = 43.757 - 0.357x), where x is the percentage of DNA and y is the sperm concentration. The linear regression line fitted to the data shows an inverse connection. The p-value is less than 0.001, showing that the correlation is statistically highly significant, and the correlation coefficient (r) is -0.41, indicating a moderately negative correlation. This result suggests that sperm concentration tends to decrease as DNA fragmentation increases.

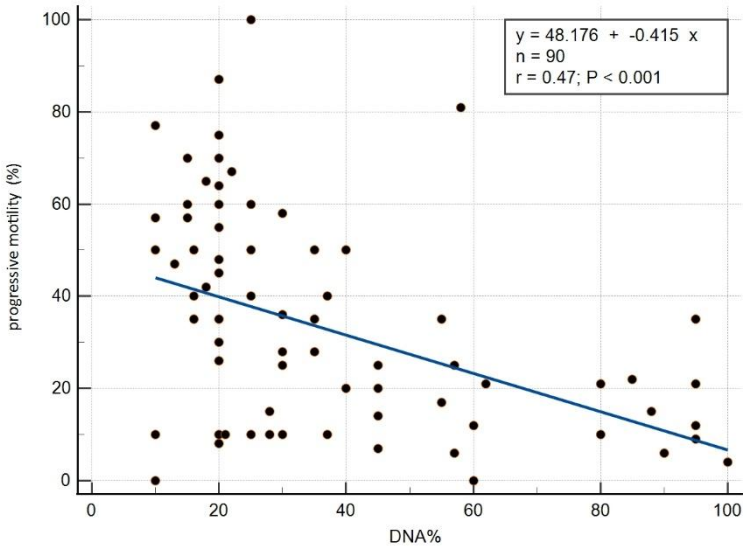

Figure (2) The correlation between DNA integrity of seminal plasma and progressive motility Percent.

Figure 2 Shows how sperm motility in seminal plasma samples is related to DNA integrity, which is given as DNA fragmentation percentage (DNA%). These two factors have a statistically significant negative association. The regression equation is (y = 48.176 - 0.415x), where x is the percentage of DNA and y is the sperm motility. The linear regression line fitted to the data shows an inverse connection. The p-value is less than 0.001, showing that the correlation is statistically highly significant, and the correlation coefficient (r) is -0.47, indicating a moderately negative correlation. This research suggests that sperm motility tends to diminish as DNA fragmentation rises.

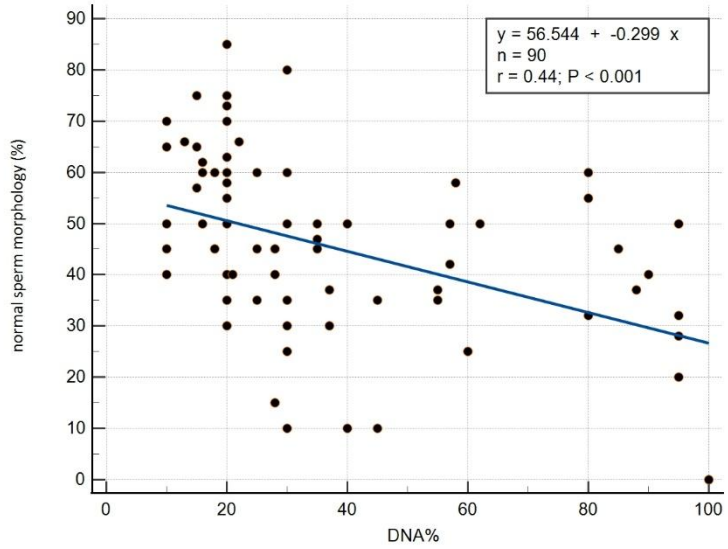

Figure (3) The correlation between DNA integrity of seminal plasma and normal sperm morphology

Figure 3 Shows how sperm morphology in seminal plasma samples relates to DNA integrity, which is represented as DNA fragmentation percentage (DNA%). These two factors have a statistically significant negative association. The regression equation is expressed as (y = 56.544 - 0.299x), where x is the percentage of DNA and y is the sperm morphology. The linear regression line fitted to the data shows an inverse connection. The p-value is less than 0.001, showing that the connection is statistically significant, and the correlation coefficient (r) is -0.44, indicating a moderately negative correlation.

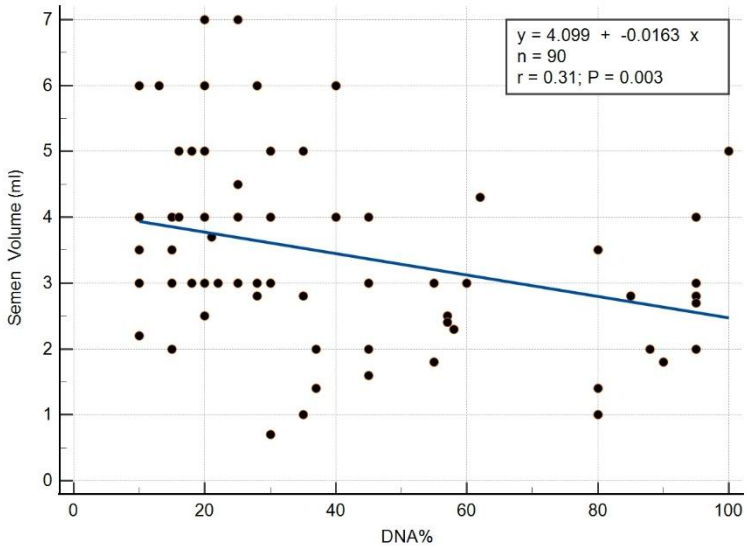

Figure (4) The correlation between DNA integrity of seminal plasma and semen volume

Figure 4 Shows how the amount of sperm (measured in milliliters) and DNA integrity (represented as DNA fragmentation percentage, or DNA%) relate to each other in seminal plasma samples. There is no discernible relationship between these two characteristics. The regression equation is expressed as follows: y = 4.099 - 0.0163x, where x is the percentage of DNA and y is the volume of sperm. The p-value is 0.003 And the correlation coefficient (r) is 0.31, indicating a weak association that is not clinically significant.

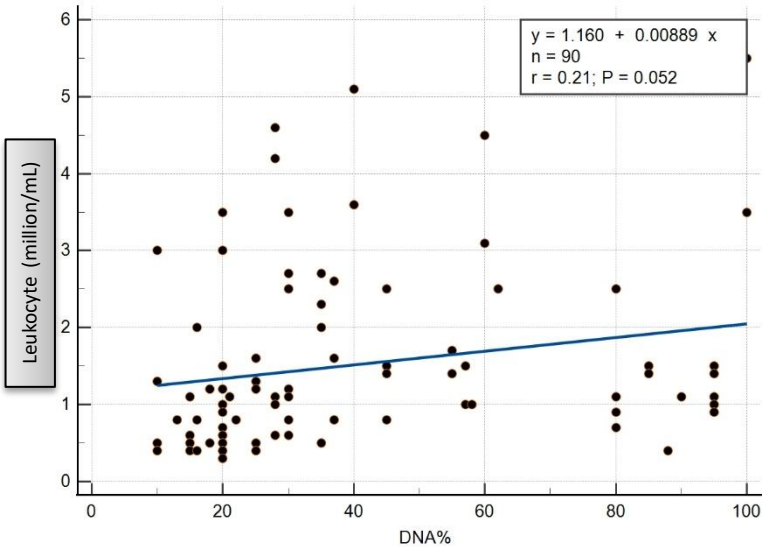

Figure (5) The correlation between the DNA integrity of seminal plasma and leukocytes**.**

Figure 5 Shows how leukocytes (measured in millions/mL) and DNA integrity (represented as DNA fragmentation percentage, or DNA%) relate to each other in seminal plasma samples. These two factors have a statistically significant positive association, as indicated by the regression equation (y = 1.160 - 0.00889x), where y represents leukocytes and x represents DNA%. The linear regression line fitted to the data shows a direct association. A moderately positive connection is shown by the correlation coefficient (r) of 0.21, and a statistically significant positive correlation is indicated by the p-value of 0.052. This study shows that when leukocytes increase, the DNA damage increases.

Discussion

The current study presents that among male infertility, there is a substantial correlation between sperm DNA fragmentation and semen quality measures. Men with abnormal semen characteristics had a higher DFI than men with normal semen characteristics, according to the results. This result is in line with earlier research that presents spermatogenesis and chromatin remodeling abnormalities, which both lead to decreased male fertility, and are reflected in high DNA fragmentation [12-13]. Sperm concentration and DNA damage were shown to be strongly inversely correlated, suggesting that higher DNA damage is linked to lower sperm concentration. Defective testicular function and increased oxidative stress both affect sperm production and genomic integrity [14-15].

There was an inverse relationship between DFI and increasing motility. Decreased mitochondrial activity, which is important for sperm motility and energy generation, frequently coexists with increased DNA fragmentation [16-17]. High DNA fragmentation also negatively impacted normal sperm morphology. These are frequently connected to abnormalities in chromatin condensation and apoptotic events during spermatogenesis, resulting in DNA packing and structural instability [18-19]. There was a correlation between leukocyte and DFI, suggesting that oxidative stress and consequent DNA damage may be facilitated by immune cell infiltration or subclinical genital tract inflammation [20-21].
Lastly, a statistically significant negative correlation between DFI and semen volume. Semen volume is not a primary sign of fertility; it

may be a good indicator of accessory gland activity, and its reduction may be linked to oxidative or inflammatory alterations in the seminal tract [22-23]. The results of this study suggest the possible advantages of making DNA fragmentation testing an additional diagnostic technique to the routine evaluations of male infertility.

Conclusion

The current study demonstrates that the integrity of sperm DNA differs among individuals with normal and abnormal semen characteristics. Decrease sperm count, motility, and normal morphology were all significantly correlated with a high DNA fragmentation index. These results suggest that DNA fragmentation is a sensitive indicator of impaired production and function of sperm. The higher DFI seen in semen samples with abnormal morphology and low motility, both with oxidative stress and abnormal chromatin package, all support the pathophysiology of male infertility. The importance of including DNA damage analysis in the routine evaluation of male infertility is shown by the strong correlation found between DNA fragmentation and semen quality. These results indicate that further study is necessary to elucidate the underlying biological mechanisms and investigate the potential therapeutic applications of DFI as a biomarker for diagnosis and prognosis in reproductive medicine.

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التحليل المقارن لمؤشرات جودة السائل المنوي وتفتت الحمض النووي في الذكور الخصيبين وغير الخصيبين

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الكلمات المفتاحية: العقم عند الذكور، كريات الدم البيضاء، حركة النطف، تجزئة الحمض النووي للنطف، تحليل السائل المنوي.

الملخص

هدفت هذه الدراسة إلى تقييم العلاقة بين تفتت الحمض النووي للنطف ومؤشرات جودة السائل المنوي لدى الذكور المصابين بالعقم. شمل الفحص 90 عينة سائل منوي، 30 عينة من ذكور مصابين بالعقم، و60 عينة من ذكور مصابين باضطرابات في معايير السائل المنوي. حُلِّل السائل المنوي وفقًا لمعايير منظمة الصحة العالمية (2010)، والتي تشمل عدد كريات الدم البيضاء، والشكل، والحركة، وتركيز الحيوانات المنوية. قُيِّم تلف الحمض النووي باستخدام طريقة تلطيخ صبغة الأنيلين الزرقاء، وصُنِّفت العينات بناءً على مدى تفتت السائل المنوي. أظهرت جميع العوامل المُحلَّلة فروقًا ذات دلالة إحصائية (P<0.001)، وفقًا للنتائج. كان متوسط تركيز الحيوانات المنوية في المجموعة السليمة أعلى بكثير، حيث بلغ 54.93±13.44 مليون/مل، مقارنةً بالمجموعة غير السليمة، والذي بلغ 17.10±15.24 مليون/مل. وبالمثل، كانت الحركة التقدمية للحيوانات المنوية في مجموعة السائل المنوي الشاذ أقل بكثير (19.53±15.30%) مقارنةً بالمجموعة الطبيعية (56.43±16.85%). وكان شكل الحيوانات المنوية في المجموعة الطبيعية (61.67±13.08%) أفضل من شكل الحيوانات المنوية في المجموعة غير الطبيعية (36.33±14.25%). بالإضافة إلى ذلك، كان حجم السائل المنوي في المجموعة الطبيعية (3.85±1.18 مل) أكبر بكثير من حجمه في المجموعة غير الطبيعية (3.26±1.47 مل). علاوة على ذلك، كان تركيز الكريات البيضاء في مجموعة السائل المنوي الشاذ (1.92±1.21 مليون/مل) أعلى بكثير من تركيزه في المجموعة الطبيعية (0.68±0.28 مليون/مل). وفقًا للنتائج، أظهرت العينات غير الطبيعية تلفًا في الحمض النووي (DNA) أكبر بكثير (49.50 ± 27.50) مقارنةً بالعينات الطبيعية (19.10 ± 5.31). وكان تلف الحمض النووي للحيوانات المنوية أعلى بكثير في المجموعة غير الطبيعية منه في المجموعة الضابطة (P < 0.001). وكان هناك ارتباط سلبي ذو دلالة إحصائية بين تفتت الحمض النووي وحركة الحيوانات المنوية (r = -0.47، P = 0.001)، والشكل (r = -0.44، P = 0.001)، والتركيز (r = -0.41، P = 0.001). وكان هناك أيضًا ارتباط إيجابي إلى حد ما بين تفتت الحمض النووي وكريات الدم البيضاء (r = 0.21، P = 0.052). وجدت هذه الدراسة أن جودة السائل المنوي المنخفضة لدى الرجال المصابين بالعقم ترتبط ارتباطًا وثيقًا بزيادة تفتت الحمض النووي للنطف، مما قد يكون مؤشرًا جيدًا لتشخيص العقم عند الذكور.