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The Role Of Sperm DNA Fragmentation On Male Infertility In Al Kut City

[1]Othman Hashem Mohammed; [2]Rania Sabri Hasan; [3]Ghassan Jabbar Khalaf; [4]Haidar Hussein Essa; [5]Luma Hikmat Kareem; [6]Wisam Basim Al-Tmemy; [7]Zeena Mohammed Abdul Hassan

[1]Embryology - kut University College; [2]Microbiology- Central Technical University-Technical Institute-Kut; [3]Parasitology- kut University College; [4]Microbiology - kut University College; [5]Microbiology- kut University College; [6]phsiology - kut University College; [7]Molecular Biology- kut University College , Wasit –IRAQ

[1]Othmanh78@gmail.com; [2]raniaalkuraishi@gmail.com; [3]Ghassan.jabbar@alkutcollege.edu.iq; [4]Haidar.Hussein@alkutcollege.edu.iq; [5]Lumahikmat@uowasit.edu.iq; [6]Pad.24.018@alkutcollege.edu.iq; [7]PESSD.24.025@alkutcollege.edu.iq

Abstract. *Infertility is one of the most common diseases in the world in last period of all types, whether it is due to (asthenospermia,oligoasthenospermia,teratospermia,hormonal imbalance). Study aims to reveal negative effect of sperm DNA fragmentation on fertilization, this study was conducted in the Al-Jazeera Specialized Laboratory in the city of Kut for the period between (January to November from 2024), two hundred A person suffering from infertility. The semen was examined for all of them, they were divided into eighty of them being normal (the control group) and one hundred and twenty suffering from infertility (asthenospermia) hormonal test and sperm DNA fragmentation test (by Kit methods) was performed for everyone , this study showed Following, we obtained higher proportions of sperm DNA fragmented infertile Which had an abnormal shape and low motility. The results are similar to those of other studies, which found that the DFI in infertile group was significantly higher than that in fertile group (34.53 ± 4.6 % compared to11.91 ± 4.0 %) and mean sperm concentration in fertile group was significantly higher compared to infertile group (83.22 ± 14.9 /ml compared to11.67 ± 10.7 /ml) from this study. Through this study, the DFI test they can be used for discrimination and between the infertile male and normal male . Male men with sperm (DFI ≥ 25.1%) had a (2.52) times higher risk of infertility compared to males with sperm (DFI < 25.1%). This study shows that there is a negative relationship between (spem DNA fragmentation , sperm motility and formation) in infertile males. In addition, it can be concluded that sperm DNA fragmentation is very useful in selecting and choosing suitable sperm to be used in many techniques, including assisted reproductive techniques. It can also be concluded that the results of sperm DNA fragmentation analysis are encouraging and useful and can be used for diagnostic purposes in determining male infertility*

Keywords: *DFI, SCD ,IVF ,LH .FSH, DFI*

Introduction

Infertility is the inability of a man to achieve pregnancy with a fertile woman after a period of one year of (unprotected) intercourse, while fertility is the state of fertility or the ability to reproduce. In general, the male partner is one of the reasons or the only reason contributing to about 39% of infertility cases. and unknown cause constitute the rest , Sperm DNA fragmentation is the presence of abnormal genetic material within the sperm cells (the presence of separation or

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interruption in the genetic material)[1]. If the genetic material within sperm cells is damaged, healthy fertilization cannot occur. DNA breaks cannot be detected in traditional semen testing, so this test is needed to more advanced technology, DNA fragmentation in sperm is one of the important and main factors that poses a risk to fertility in men, infertility affects about (15%) of men and women of reproductive age [2]. Men Infertility represents about 30-40% of infertility cases between couple. One out of every eight married people faces problems when trying to have a first child, and one out of every six people faces problems when trying to have a second child[2]. The inability to conceive and have children affects the morale of both partners and may lead to depression [3].

Infertility or poor fertility motivates couples, men and women, to seek treatment because the disease may affect the behavior of couples in general. The natural human desire is to procreate and have children for the purpose of continuing and reproducing. There are 3% of women who are not have child, Also, about 5% women do not have the ability to give birth to the number of children they want [4]. Fertility in men can decrease as a result of varicocele, endocrine disorders, acquired or congenital malformations of the genitourinary system, malignant tumors, high scrotal temperature, reproductive system infections, genetic abnormalities, and also immune factors. In addition, genetic abnormalities affect about 15% of males suffering from infertility, including chromosomal aberrations and individual genetic mutations[5]. Male, female, or both partners can be the cause of infertility, there are unknown reasons for many cases of infertility in the world. It is very important to conduct medical examinations for people about to get married in order to avoid illness. Methods of examinations and evaluation, assisted reproductive techniques and appropriate IVF procedures can help provide better chances and hope to infertile partners[6].

The male partner must undergo a seminal fluid analysis as a basic procedure and main examination. If only the male is sterile, time can be saved, procedures and treatments can be speeded up and the cost can be reduced as well. One of the main and important factors that have a negative impact on infertility in men is broken or fragmented DNA in sperm.

Infertility in men is problems, disorders and changes, the known causes of which represent about 30-45%, and other causes and problems are not known yet [7].

It is very important to check for DNA damage on sperm because the father provides half of the DNA and the mother the other half in human sperm. For the purpose of functional and anatomical integration of the complex reproductive system, male sperm are formed in the germinal

epithelium and as a result of the organizational role of the hypothalamic-pituitary testis axis they become highly sensitive; Its changes become evident even when fertility deteriorates [8]. FSH controls sperm formation and maturation by supplying to both the Sertoli cells and the germinal epithelium, LH hormone is responsible for the formation and synthesis of sperm, as this protein regulates the synthesis of the male hormone testosterone in the accessory tubular Leydig cells [9].

To achieve accurate detection of the causes of infertility in men, the sperm DNA of married men must be intact and unfractionated. The main important causes of early pregnancy loss are genetic deformities, disorders and mutations in men's sperm. One of the most important diagnostic tests used for this purpose is an accurate, rapid, and diagnostic test that is based on the dispersion of sperm chromatin, normal sperm create halo zones around the DNA[10]. It has been found that sperm quality will decrease significantly and clearly if sperm DNA fragmentation in men exceeds 30%. Therefore, this percentage can be considered as a guide for the purpose of choosing the appropriate treatment and technique to treat infertility in men.

DNA damage in sperm cells was studied for a group of infertile males, one hundred and twenty patients with abnormal indices sperm (asthenospermia and oligospermia), and a group of eighty patients with normal indices semen to find out whether the sperm count had decreased or not. (SCD) analysis may augment the information and indicators obtained through routine sperm analysis to identify, clarify and treat the causes of infertility[11,12].

Aim of the study

This study aims to know the role and negative impact of DNA fragmentation on fertility among men who suffer from the inability to achieve pregnancy with fertile women in the city of Kut.

Patients, Materials and methods.

Patients,

Two hundred patients were analysed, One hundred and twenty patients in (action group) and eighty patients in the (group control). All the patients who were analyzed were from the city

of Kut, Wasit Governorate. The period sampling was from January to February 2024. All tests were conducted at Al-Jazeera Specialized Laboratory in the city of Kut, in accordance with World Health Organization guidelines (WHO). All sperm tests were performed SFA, DNA Fragmentation in sperm and FSH, LH, prolactin, testosterone hormones levels analysis was performed by ESIA. The data obtained were compared between the two groups by one-way (ANOVA) test, which is mean (± SD). Also, a p value (<0.05%) was considered statistically significant in the research.

Seminal fluid analysis.

Semen samples were collected from patients suffering from infertility after informing them of this. (This is within the ethics of scientific research). Semen samples were collected after abstaining from sexual intercourse for a period of 3-5 days and were placed directly in a clean, dry container and immediately marked (name and laboratory number). The samples were then examined individually using macroscopic and microscopic examinations according to the standard form of the World Health Organization manual (1999 and 2010)[13].

Microscopic examination

After ensuring that the semen sample became liquid(normal viscosity), the sample was mixed well, then a drop of 10 microliters of the sample was placed on a warm glass slide (37 degrees Celsius) and then covered with a standard cover (22 x 22 mm). After that, the sample was examined under an optical microscope using a standard optical microscope. And use the 40X magnification power of the objectives. Semen samples were evaluated for the following sperm function parameters recommended by the World Health Organization (18,19) namely: sperm concentration in semen (million/ml), sperm motility in semen %, normal and abnormal sperm in Semen morphology, as well as the number of inflammatory round cells (cell/HPF) (%) [14.15].

Sperm DNA fragmentation

This examination was done using a special kit

-Principle

Unfixed intact sperm were placed on a slide pretreated in an inert micro-agarose gel. The initial acid treatment leads to a change in the nature of the DNA in sperm cells taken from infertile men with fragmented DNA. The lysis solution then removes most of the nuclear proteins. In the absence of significant DNA breakage in sperm cells, nucleoids with fragmented DNA also show no scattering halo or the halo is minimal[16.17].

$$\text{SDF (\%)} = \frac{\text{Fragmented + degraded}}{\text{Total cells quantified}} \times 100$$

Hormonal tests

The hormone test was performed in the usual manner, whereby a sample of the patient's blood was drawn after he sat on the blood drawing chair. The patient's arm was sterilized from the area from which blood was to be drawn. 5 ml of the patient's blood was taken and placed in a sterile test tube. After that, the centrifugation process was performed using a centrifuge. For the purpose of obtaining blood serum, each test of the hormones required to be performed is performed (Kit method according to procedure), and the ELISA device is used to calculate hormone values[18,19].

Statistical analysis

Data for the current study obtained in the research, they were expressed as (Mean±SEM) and the data obtained were analyzed using analysis of variance (ANOVA). When the F value reaches the significant level, also in this study, the (LSD) the least significant test was used to compare the results of the study. Also, (P values <0.05) were considered statistically significant [20].

Results

The results obtained after performing the analysis and comparing the hormonal parameters ,Seminal fluid examination and sperm DNA Fragmentation in both groups of patients (the work group and the control group) The following results were obtained.

The mean values of sperm concentration in fertile men (83.22 ± 14.9) were significantly higher (P<0.001) than those in infertile men(11.67 ± 10.7). Also, the mean values of hormone levels (LH,FSH.Prolactin) (14.14 ± 4.9),(17.35 ± 8.1),(26.56 ± 4.6) respectively in infertile men were significantly higher (P<0.001) than those in fertile men(5.98 ± 3.1),(3.42 ± 4.1),(6.29 ± 8.9). In

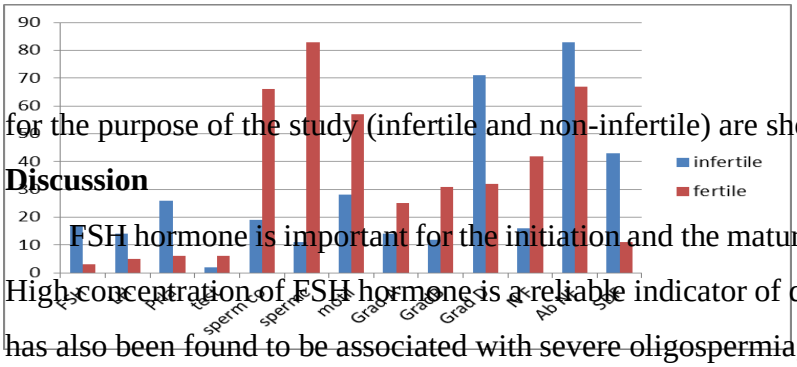
this study, we found that the average level values of the male hormone testosterone (6.88 ± 4.6) in fertile men were significantly higher (P<0.001) than those in infertile men, as the relationship was directly related between the levels of the male hormone testosterone and sperm concentrations in fertile men, as shown in the table (1). In addition, the average movement values (A,B and C) respectively (25.21 ± 7.4),(31.73 ± 10.0) in fertile men were significantly higher (P<0.001) than those in infertile men(14.09 ± 10.8),(12.05 ± 10.1), as the relationship was directly related to male hormone levels. In Table (1) it was found that the average sperm DNA fragmentation values in fertile men(11.91 ± 4.0) were significantly lower (P < 0.001) than those in infertile men(34.53 ± 4.6). The relationship was inverse, especially with the movement and shape of sperm in fertile men, in this study, the average values of both shape and motility of sperm in fertile men were significantly higher when compared to infertile men, and this is inversely proportional to the average values of sperm DNA fragmentation, as shown in table no (1).

Table 1. In this table, the results of patients suffering from infertility for the two groups taken for the purpose of the study (infertile and non-infertile) are shown.

Test	Infertility- (group) 120 patients	Fertile- (group) 80 patients	Significant- (P < 0.05)
FSH-H	**(17.35 ± 8.1)**	**(3.42 ± 4.1)**	**p<0.0001**
LH-H	**(14.14 ± 4.9)**	**(5.98 ± 3.1)**	**p<0.01**
Prolactin-H	**(26.56 ± 4.6)**	**(6.29 ± 8.9)**	**p<0.0001**
Testosterone-H	**(2.16 ± 4.9)**	**(6.88 ± 4.6)**	**p<0.0001**
Sperm count/million	**(19.67 ± 19.7)**	**(66.43 ± 34.4)**	**p<0.0001**
Sperm concentration	**(11.67 ± 10.7)**	**(83.22 ± 14.9)**	**p<0.0001**
Motility	**(28.29 ± 18.3)**	**(57.15 ± 10.8)**	**p<0.0001**
Grade (A)	**(14.09 ± 10.8)**	**(25.21 ± 7.4)**	**p<0.0001**
Grade (B) and (C)	**(12.05 ± 10.1)**	**(31.73 ± 10.0)**	**p<0.0001**
Grade D	**(71.59 ± 18.3)**	**(32.7 ± 10.9)**	**p<0.0001**
Normal form	**(16.90 ± 14.4)**	**(42.6 ± 15.2)**	**p<0.0001**

Abnomal form	**(83.09 ± 14.4)**	**(67.8 ± 16.4)**	**p<0.0001**
(SDF %)	**(34.53 ± 4.6)**	**(11.91 ± 4.0)**	**p<0.0001**

The results of patients suffering from infertility for the two groups taken for the purpose of the study (infertile and non-infertile) are shown



Discussion

FSH hormone is important for the initiation and the maturation of sperm in the infertile male. High concentration of FSH hormone is a reliable indicator of damage to the seminal epithelium. It has also been found to be associated with severe oligospermia, the results also showed that higher levels of FSH in the blood correspond to an increased severity of damage to the seminiferous epithelium. FSH, LH, and testosterone are good indicators of infertility in men[21,22].

In this study, FSH, LH hormone levels elevated of were found in the blood serum of males with oligospermia and azoospermia compared to those with normal sperm. It has also been observed that high levels of FSH, LH, testosterone hormones influence the decline of sperm indice in males[23.24].

LH hormone stimulates indirectly spermatogenesis via testosterone, while FSH hormones directly acts on the seminiferous tubules. Additionally, FSH has a major role in stimulating meiotic DNA synthesis of male sperm[24,25]. High levels of gonadotropin hormone in the blood serum may cause abnormalities in sperm formation, which may lead to a decrease in sperm count and thus infertility. In this study, we obtained high percentages of fragmented DNA in infertile sperm that had low motility and abnormal shape[26.27]. These results are similar to studies (Evean et al., 2015)[28]. Which hit that the DFI in fertile group was significantly lower than that in infertile group. Through this

study, the DFI test can be used as a guide to identify and distinguish infertile men from normal men[29,30].

Conclusion, in this study a negative relationship indicates between DNA fragmentation and sperm movement (motility) and shape (morphology) in infertile males. From our findings, we can consider that DNA fragmentation in the sperm of infertile men is a very useful guide to knowing which sperm are good and which can be used for treatment in assisted reproductive techniques and also a guide to knowing the appropriate treatment.

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