Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(4): 985-988
doi: 10.52142/omujecm.39.4.12

The effects of sperm parameters and sperm DNA damage on pregnancy outcomes of women undergoing intrauterine insemination

Murat ÖNAL[1], Müstecep KAVRUT[2], Nur DOKUZEYLÜL GÜNGÖR[3], Tuğba GÜRBÜZ[4*]

[1]Gynolife IVF Clinic, Lefkosa, Cyprus
[2]Private Clinic,İstanbul, Türkiye
[3]Department of Obstetrics and Gynecology IVF Clinic Bahçeşehir University Göztepe Medikal Park Hospital İstanbul, Türkiye
[4]Department of Obstetrics and Gynocology, Medistate Hospital, İstanbul, Türkiye

Received: 16.07.2022 • **Accepted/Published Online:** 20.07.2022 • **Final Version:** 29.10.2022

Abstract

In this study, we compared the effects of sperm parameters and sperm DNA damage on pregnancy outcomes of women undergoing intrauterine insemination (IUI) because of an ovulatory dysfunction (OD) and unexplained infertility (UEI). This study was retrospective, and semen samples were collected from records of 88 infertile couples referred to private clinic for infertility treatment from December 2019 to March 2020. The study has two groups: Groups 1: couples with UEI, and Group 2: fertile males with their partners having OD. The participants' age and body mass index (BMI) were 28.20 ± 3.08 and 25.45±2.25, respectively. In the control group, the pregnancy rate was 9/41 (%21.9), and one out of nine patients had a miscarriage. The pregnancy rate in the study group (UEI) was 8/47 (17%), and half of the pregnancies ended as miscarriage. Our results showed that sperm DNA damage increases the abortion rate but has not influenced the pregnancy rate in IUI.

Keywords: sperm DNA damage, pregnancy, unexplained infertility, intrauterine insemination

1. Introduction

According to the report of the World Health Organization, pregnancy failure has affected more than 80 million people in the world (1,2). The origin of infertility can be due to male or female factors or both. Based on this, 40% of male factors, 40% of female factors, and 20% of both male and female factors are involved in infertility (2). Many factors like age, autoimmune diseases and body mass index can affect the sperm parameters (3, 4).

Spermatozoa of infertile men often have different functional and structural defects (5). Standard analysis of semen, which includes concentration, motility, and sperm morphology, is considered a sensitive biological marker. However, these markers cannot give us information about the health of the genetic material of male gametes and cannot be used as predictors of fertility ability (5). Several tests have been introduced based on the physiological and molecular function of sperm in the fertilization process, including the ability of sperm to attach to the transparent layer around the egg in the first stage of fertilization, the chemical penetration power of sperm, and examining the state of DNA damage (5-8).

Sperm makes up half of the genetic material of the embryo resulting from fertilization, so half of the percentage of fertility success depends on the flawless transfer of sperm DNA during its journey from the testicle to the fallopian tubes in women (7). Sperm DNA damage can be caused by DNA fragmentation, improper chromatin packaging, and epigenetic defects (8, 9). Clinical evidence shows that sperm DNA damage has harmful and destructive effects on fertility results, and the amount of these damages is much higher in infertile men than in fertile men (5,6). In addition, various studies indicate that DNA fragmentation can have a negative effect on sperm parameters and the rate of pregnancy (6-9).

Defects in sperm chromatin structure are typically associated with abnormal content of nuclear proteins or DNA strand breaks (10), which are detected using different techniques such as Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), Sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) test, aniline blue,acridine orange (AO) and Chromomycin A3 (CMA3) (7, 8, 10, 11).

The first step for infertility treatment in many couples is the treatment of intrauterine sperm insemination (IUI). This method is less invasive and less expensive than other assisted reproductive treatment methods.

In this study, the sperm parameters and DNA damage of patients in two groups are retrospectively compared to investigate their effects on pregnancy outcomes.

*Correspondence: drtgurbuz@hotmail.com

2. Materials and Methods

This study was retrospective, and semen samples were collected from records of 88 infertile couples referred to private clinic for infertility treatment from December 2019 to March 2020. The Ethics Committee of Beykoz University approved this retrospective study (Decision no: 1 Date: 26.10.2020). The sperm parameters and DNA damage of subjects are compared to investigate their effects on pregnancy outcomes. The study has two groups: Groups 1: Couples with unexplained infertility (UEI), and Group 2: fertile males with their partners having ovulatory dysfunction (OD). The inclusion criteria were: (1) Men between the ages of 25 and 40. The exclusion criteria were: (1) Absence of diabetes, thyroid dysfunction, and systemic diseases. (2) Men with systemic disease and known history of varicocele are excluded. A total of 47 people in the first and 41 in the second groups were included. All parameters related to participants' sperm were extracted from electronic records and analyzed. After 3-5 days of sexual abstinence semen analyses were performed. Aniline blue staining was used to determine sperm DNA damage as described in the literature (8).

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the groups' non-normality before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, including age, body mass index (BMI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, prolactin (ng/ml), estradiol, spermiogram parameters: volume, numbers (Millions), mobility (%). The Mann-Whitney test was performed to study the difference between the two groups. SPSS v22 was used for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

Data analysis was performed on SPSS 21 (SPSS Inc., IBM, Armonk, NY, USA). Normality of distribution was evaluated with the Shapiro-Wilk test. Normally distributed variables were analyzed with the independent samples t-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Spearman correlation coefficients were calculated for the assessment of relationships between continuous variables. The distributions of categorical variables were evaluated using Pearson Chi-square tests or Fisher's exact tests. Logistic regression analysis (backward conditional method) was performed to determine risk factors affecting fertility status. Data were given as mean ± standard deviation for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables. Differences were considered statistically significant if the p-value <0.05. To calculate the sample size with the G-Power 3.1 (http://www.gpower.hhu.de/) program, two groups' total mean was measured based on the Mann-Whitney test with the power of 95%, effect size of 50%, and 0.05 type 1 error for at least 92 patients (12).

3. Results

This study was conducted on 88 males in two groups: Case-Couples with UEI who underwent IUI, and control- fertile males with their wife having OD. The average age of males and females and the BMI of both groups were not significantly different. The demographic and laboratory characteristics of both groups are shown in Table 1.

Table 1. Demographic and laboratory characteristics of both groups

Study parameters	Case-UEI (n=47) Mean±SD	Control-OD (n=41) Mean±SD	*p-value*
Male age	33.17±3.33	33.1±3.92	0.977*
Female age	30.02±3.48	30.05±3.09	0.969**
Male BMI	24.61±1.78	24.68±1.52	0.651*
Duration of infertility ±year	3.26±1.47	3.22±1.06	0.976*
FSH	5.95±2.48	4.76±1.42	0.027*
LH	4.46±1.64	4.52±1.37	0.587*
Total testosterone ±ng/ml	5.8±2.69	4.68±1.28	0.062*
E2 ±pg/ml	17.61±5.43	14.88±2.06	0.060*
Prolactin ±ng/ml	13.27±3.56	14.47±2.09	0.074*
Varicocele	0±0%	0±0%	0.862***
Cigarette	10±24.4%	24±51%	**0.036*** **
Alcohol	41±100%	45±95.7%	**0.025*** **

* Mann-Whitney test **Independent t-test ***Pearson Chi-square test

No significant difference was observed in the variables duration of infertility, FSH, LH, total testosterone, E2, prolactin and varicocele between the two groups.

An interesting result was the significantly higher consumption of cigarettes and alcohol in the control group, whose patients partners had infertility complications, and the participants themselves had no fertility complications (p=0.036 and p= 0.025). Participants in the case group (UEI) who had infertility problems themselves had significantly less alcohol and cigarette consumption than the control group. Table 2 shows the comparison of sperm characteristics of the two groups.

Table 2. Comparison of sperm characteristics of two groups

Sperm quality parameters	Case-UEI (n=47) Mean±SD	Control (n=41) Mean±SD	*p*
Sperm count ±/mL	33.92±24.49	35.73±19.85	0.351*
Total motility	59.3±15.89	61.73±9.53	0.870*
Sperm morphology	1.74±0.71	2.2±0.68	**0.006***
DNA damage ±%	38.19±15.55	25.15±5.69	**<0.001***

* Mann-Whitney test

There was no significant difference in sperm count and total motility between the two groups. Also, the sperm morphology and DNA damage were significantly different between the two groups (p-value= 0.006 and p-value= 0.001). Fig. 1 shows the information related to pregnancy and abortion in two groups. In the control group, the pregnancy rate was 9/41 (21.9%), and one out of nine patients miscarried. The pregnancy rate in the study group was 8/47 (17%), and half of the participants miscarried.

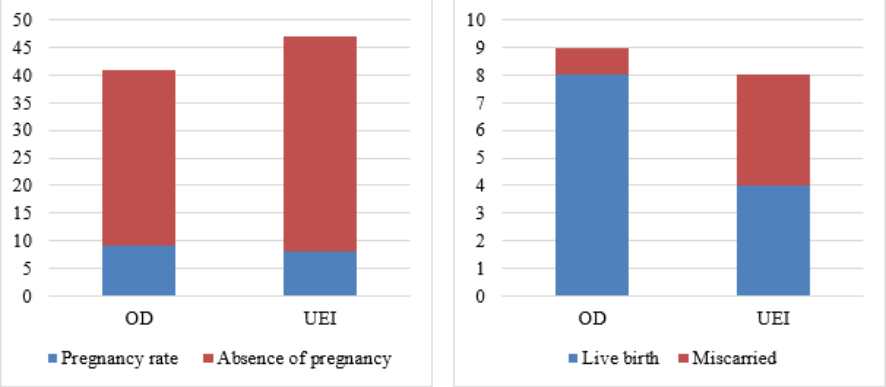

Fig. 1. Information related to pregnancy and abortion in two groups

4. Discussion

15-20% of couples are infertile despite trying to have children, which is the cause of half of the infertility cases due to the male factor. The majority of male infertility is associated with abnormal sperm parameters (11). Therefore, these people are candidates for assisted reproductive methods and may have experienced these treatments many times. One of the essential factors in the success of gamete fertilization is the health of sperm DNA and chromatin. Various studies show that the lower the quality of these sperm parameters, the more problems the sperm DNA health faces (10,12-16). Therefore, before choosing the appropriate treatment method, evaluating the amount of sperm DNA damage is recommended (16). In many of these cases, if there is damage to sperm DNA, it is possible to improve sperm quality by performing appropriate therapeutic interventions. Ensuring the health of sperm DNA is one of the most critical things in the egg fertilization process, the continuation of embryo development, and the success of assisted reproductive methods (17).

Therefore, sperm DNA health is one of the most critical characteristics of sperm, and its evaluation can provide valuable information regarding fertility ability (18). Therefore, in the present research, we investigated the effect of DNA damage on fertility and abortion rates. In this study, patients used IUI as a treatment method. Our results showed a significant difference in sperm DNA damage between the two study groups and the control group, which is comparable to the results of previous studies in this field (15-19).

This study showed a significant relationship between DNA damage and abnormal sperm morphology. These results agree with the previous research that there is a significant relationship between semen parameters and DNA damage (20-22). Therefore, it can be said that the sperm of infertile people with abnormal morphology probably have more DNA damage compared to fertile people with normal seminal fluid parameters. Also, considering that this relationship is statistically significant but has a low correlation coefficient, it cannot be concluded that every sperm that is normal in terms of morphology is also healthy in terms of genetic material or aneuploidy. Therefore, some people have normal sperm parameters but different degrees of DNA damage, which can cause much UEI (20). In addition, sperm DNA damage is probably more affected by improper chromatin packaging than sperm morphology abnormalities during spermatogenesis (21).

This study showed a significant relationship between DNA damage and vitamin deficiency. These findings indicate that vitamin deficient sperm are more susceptible to DNA damage. The results obtained from our study are comparable to those of previous studies in this field (15, 22, 23).

The effect of DNA damage on the pregnancy rate is conflicting among different studies (14,16, 21,24). There are many reasons for these contradictions, which can be related to factors such as the sperm preparation process, the fertilization process (CSI, IUI, IVF), the DNA damage evaluation method (TUNEL, SCSA, SCD, AO, COMET), and the way the test is performed (manual, automatic). In the present study, no correlation was observed between DNA damage and the IUI pregnancy rate of the two groups, which is consistent with the results of other findings in this field (22-24). However, a significant relationship between DNA damage and increased miscarriage was observed, so sperm containing damaged DNA have more miscarriage risk. The results of this study are compatible with previous studies in this field (23-26). Despite the conflicting results presented in this field in different studies, our findings indicate that sperm DNA damage has no effect on the pregnancy rate in IUI patients of the two groups, but this factor can probably affect the miscarriage rate. The main limitation of this study was the small sample size. It is suggested to investigate the impact of DNA damage on pregnancy outcomes in a higher sample size.

Seminal fluid samples are generally heterogeneous and probably contain sperm with different defects. These defects can be related and likely affect pregnancy and the fetus's early development. Also, the method of sperm selection, based on its functional capacity, can play an essential role in advancing the treatment result. From this study and other studies, it can be concluded that the defects of the sperm DNA damage affect the abortion rate, but it has not influenced the pregnancy rate. However, the effect of these defects on babies

resulting from assisted reproductive techniques needs further study.

Conflict of interest
The authors declared no conflict of interest.

Funding
No funding was used for the study.

Acknowledgments
None to declare.

Authors' contributions
Concept: M.Ö., N.D.G., T.G., Design: M.K., T.G., Data Collection or Processing: N.D.G., M.Ö., M.K., Analysis or Interpretation: M.K., T.G., Literature Search: M.K., N.D.G., Writing:M.Ö., T.G.

References
1. Agarwal A, Panner Selvam MK, Baskaran S, Cho C-L. Sperm DNA damage and its impact on male reproductive health: a critical review for clinicians, reproductive professionals and researchers. Expert review of molecular diagnostics. 2019; 19(6): 443-57.
2. Antonouli S, Papatheodorou A, Panagiotidis Y, Petousis S, Prapas N, Nottola SA, et al. The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. Archives of gynecology and obstetrics. 2019; 300(1): 207-15.
3. Beaud H, Tremblay AR, Chan PT, Delbes G. Sperm DNA damage in cancer patients. Genetic Damage in Human Spermatozoa: Springer; 2019; 189-203.
4. Casanovas A, Ribas-Maynou J, Lara-Cerrillo S, Jimenez-Macedo AR, Hortal O, Benet J, et al. Double-stranded sperm DNA damage is a cause of delay in embryo development and can impair implantation rates. Fertility and sterility. 2019; 111(4): 699-707.
5. Chen Q, Zhao J-Y, Xue X, Zhu G-X. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. Reproductive Toxicology. 2019;86:50-5.
6. Colacurci N, De Leo V, Ruvolo G, Piomboni P, Caprio F, Pivonello R, et al. Recombinant FSH improves sperm DNA damage in male infertility: a phase II clinical trial. Frontiers in endocrinology. 2018; 9: 383.
7. Deng C, Li T, Xie Y, Guo Y, Yang Qy, Liang X, et al. Sperm DNA fragmentation index influences assisted reproductive technology outcome: A systematic review and meta-analysis combined with a retrospective cohort study. Andrologia. 2019; 51(6): e13263.
8. Güngör K, Dokuzeylül Güngör N,Basar MM, Cengiz F, Ersahin SS, Cil K. Relationship between serum vitamin D level ,semen parameters and sperm DNA damage in men with unexplained infertility Eur Rew Med Pharmacol Sci 2022; 26(2): 499-505.
9. Esteves SC. Interventions to prevent sperm DNA damage effects on reproduction. Genetic damage in human spermatozoa: Springer; 2019; 119-48.
10. Gill K, Rosiak-Gill A, Jakubik J, Patorski L, Lukaszuk M, Piasecka M. The higher risk for sperm DNA damage in infertile men. Ginekologia polska. 2019; 90(12): 684-91.
11. Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. Andrology. 2020; 8(1): 53-81.
12. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 2007; 39(2): 175-91.
13. Haddock L, Gordon S, Lewis SE, Larsen P, Shehata A, Shehata H. Sperm DNA fragmentation is a novel biomarker for early pregnancy loss. Reproductive BioMedicine Online. 2021; 42(1): 175-84.
14. Kabukçu C, Çil N, Çabuş Ü, Alataş E. Effect of ejaculatory abstinence period on sperm DNA fragmentation and pregnancy outcome of intrauterine insemination cycles: A prospective randomized study. Archives of Gynecology and Obstetrics. 2021; 303(1): 269-78.
15. Kim GY. What should be done for men with sperm DNA fragmentation? Clinical and experimental reproductive medicine. 2018; 45(3): 101.
16. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. Fertility and sterility. 2019; 112(1): 54-60.
17. Nicopoullos J, Vicens-Morton A, Lewis SE, Lee K, Larsen P, Ramsay J, et al. Novel use of COMET parameters of sperm DNA damage may increase its utility to diagnose male infertility and predict live births following both IVF and ICSI. Human Reproduction. 2019; 34(10): 1915-23.
18. Paoli D, Pallotti F, Lenzi A, Lombardo F. Fatherhood and sperm DNA damage in testicular cancer patients. Frontiers in endocrinology. 2018; 9: 506.
19. Ribas-Maynou J, Benet J. Single and double strand sperm DNA damage: different reproductive effects on male fertility. Genes. 2019; 10(2):105.
20. Ribas-Maynou J, Yeste M, Becerra-Tomás N, Aston KI, James ER, Salas-Huetos A. Clinical implications of sperm DNA damage in IVF and ICSI: updated systematic review and meta-analysis. Biological Reviews. 2021; 96(4): 1284-300.
21. Selvam MKP, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab Journal of Urology. 2018; 16(1): 65-76.
22. Sugihara A, Van Avermaete F, Roelant E, Punjabi U, De Neubourg D. The role of sperm DNA fragmentation testing in predicting intra-uterine insemination outcome: A systematic review and meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2020; 244: 8-15.
23. Tvrdá E, Arroyo F, Gosálvez J. Dynamic assessment of human sperm DNA damage I: The effect of seminal plasma-sperm co-incubation after ejaculation. International Urology and Nephrology. 2018; 50(8): 1381-8.
24. Yang H, Li G, Jin H, Guo Y, Sun Y. The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. Translational andrology and urology. 2019; 8(4): 356.
25. Zhang J, Xue H, Qiu F, Zhong J, Su J. Testicular spermatozoon is superior to ejaculated spermatozoon for intracytoplasmic sperm injection to achieve pregnancy in infertile males with high sperm DNA damage. Andrologia. 2019; 51(2): e13175.
26. Zheng W-W, Song G, Wang Q-L, Liu S-W, Zhu X-L, Deng S-M, et al. Sperm DNA damage has a negative effect on early embryonic development following in vitro fertilization. Asian journal of andrology. 2018; 20(1): 75.