CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1745498

Available online at: http://www.iajps.com Research Article

SPERM DNA FRAGMENTATION, AGE AND MALE INFERTILITY

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Abstract:

The aging process in the male reproductive system may include changes in testicular tissue, sperm production, and the like. Unlike females, males do not experience a substantial, rapid change (which occurs over a few months) until they grow older, instead of menopausal women, changes in males occur gradually (andropause). Purpose of the work: It is determined how age affects the appearance of male infertility. For the purpose of our study we analyzed: Sperm DNA fragmentation and oxidative stress. Materials and Methods: There were analyzed 257 patients, 169 patients in the working group and 88 patients in the control group. Patients received for analysis were all from the Republic of Kosovo. The sampling period was 2014/2018. All analyses were performed at Biolab Zafi, Laboratory in Peja. Results: The average DNA fragmentation values in sperm and oxidative stress were higher in the study group compared to that of the control. All differences were of significant statistical significance $P<0.05$. Conclusion: We can conclude that the results of DNA fragmentation in the sperm are encouraging and can be used for diagnostic purposes in determining male infertility. We recommend that an assessment of oxidative stress levels in infertile male ejaculation should be part of routine work in clinical andrology clinics to assist clinicians in determining infertility status and in setting optimal medical treatment.

Key words: Sperm DNA fragmentation, IVF/ICSI, SCD.

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Please cite this article in press Afrim Zeqiraj et al., ***Sperm DNA Fragmentation, Age and Male Infertility.,*** *Indo Am. J. P. Sci, 2018; 05(12).*

INTRODUCTION:

Sperm quality is usually considered to be a measure of male fertility and changes in sperm quality may occur after exposure to toxic agents or from life-cycle effects such as age (Eskenazi B, 2003, Eskenazi B, 2005). The aging process in the male reproductive system may include changes in testicular tissue, sperm production, and the like. Unlike females, males do not experience a substantial, rapid change (which occurs over a few months) until they grow older, instead of menopausal women, changes in males occur gradually (andropause) (Brawler MK, 2004). Understanding the impact of male age on fertility has become more and more prominent in public health issues. This is because a growing number of males choose to have offspring at older ages (Kovac JR, 2013). In the context of male fertility, the quality of the ejaculate is considered to be a progressive and satisfactory measure, and that changes in the quality of the sperm may occur after exposure to toxic agents or the effects of other age-related factors. Clinical studies show that age-related is associated with reduced ejaculation volume, decreased sperm mobility and with the impaired morphology of spermatozoa (Brawler MK, 2004; Kovac JR., 2013), while sperm concentration remains the highest great constants (Eskenazi B et al., 2003). It is unclear whether these researches are applicable to the general population of healthy people. Older men (eg> 50 years) are more clinically studied (Chia SE et al., 2004). In addition, the histories of patients using cigarettes and the duration of abstinence have not been studied sufficiently. In addition, males can be associated with increased exposure to certain physical and chemical substances that may adversely affect the quality of spermatozoa. For best diagnostic purposes, investigate the possible relationship between men's age on ejaculation volume, sperm concentration, morphology, and sperm motility in male infertile and male control groups. The conducted studies provided extensive information on the effect of medicines, lifestyle and workplace exposure on sperm quality.

PURPOSE OF THE WORK:

It is determined how age affects the appearance of male infertility. For the purpose of our study we analyzed: Sperm DNA fragmentation and oxidative stress.

MATERIALS AND METHODS:

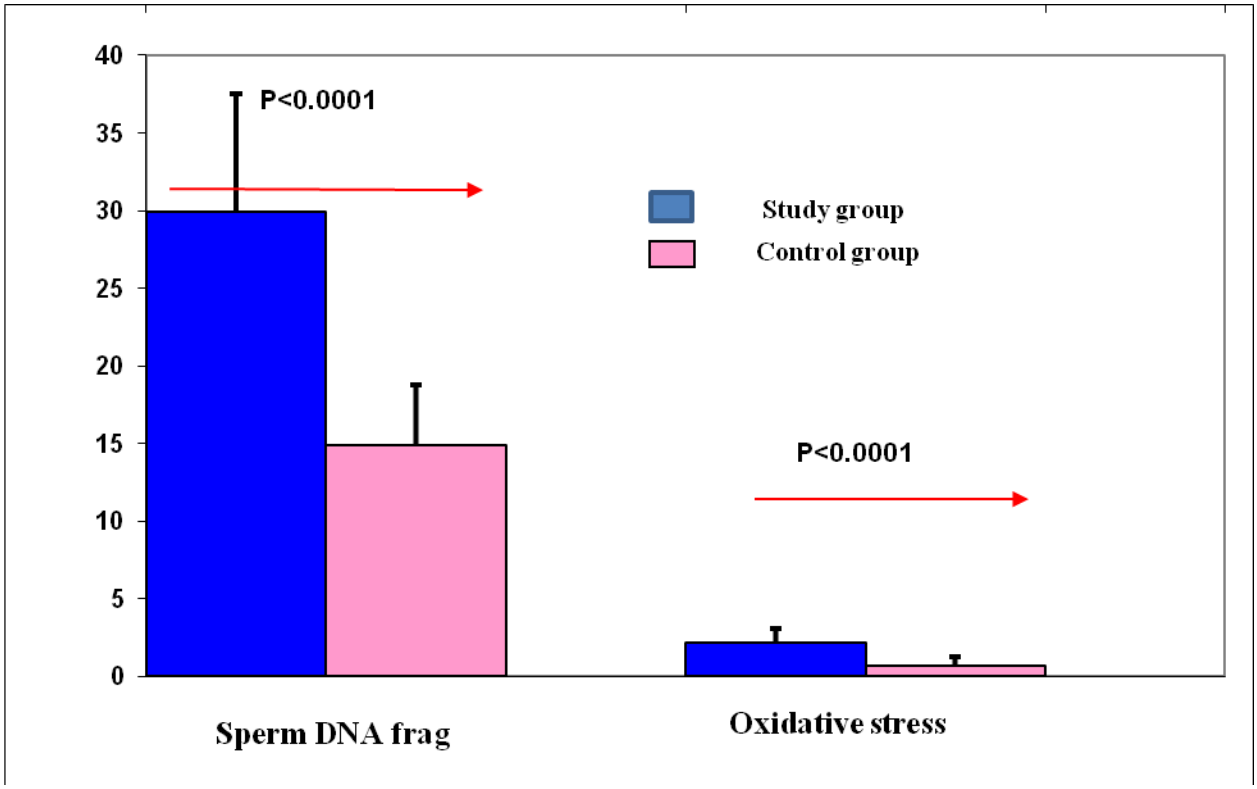
There were analyzed 257 patients, 169 patients in the working group and 88 patients in the control group. Patients received for analysis were all from the Republic of Kosovo. The sampling period was 2014/2018. All analyses were performed at Biolab Zafi, Laboratory in Peja. In the study group, there are men with asthenospermia, oligospermia, and oligoasthenospermia, whereas in the group control the normospermia males. The statistical processing of the data is done with the statistical package SPSS 22.0. From the statistical parameters are calculated the arithmetic average, the standard deviation, the minimum, and the maximum value. Qualitative data testing was done with the X2-test of quantitative data that had normal T-test distribution, while those with non-normal distribution with the Mann-Whitney test or Kruskal Wallis test. The difference is significant if P <0.05. The fragmented DNA analysis was performed according to the procedure mentioned in the instruction of the Halosperm / Halotech DNA reagent, S.L. Madrid, Spain. The oxidative stress analysis of DNA was performed according to the procedure mentioned in the instruction of the Oxisperm / Halotech DNA reagent, S.L. Madrid, Spain.

RESULTS:

Table 1. Comparison of parameters between the study group and the control group

Parameters	Study group (Mean ± SD)	Control group (Mean ± SD)	Test	P-value
N	169	88		
Sperm DNA fragmentation (%)	29.96 ± 7.56	14.91 ± 3.88	U'=14543	P < 0.0001
Oxidative stress	2.14 ± 0.98	0.69 ± 0.55	U'=1679.5	P < 0.0001

The average DNA fragmentation values in sperm and oxidative stress were higher in the study group compared to that of the control. All differences were of significant statistical significance (Table 1).



Graph 1. Comparison of parameters between the study group and the control group.

Table 2. Comparison of sperm DNA fragmentation parameters and oxidative stress between study groups

	<30 years (mean ± SD)	30-39 years (mean ± SD)	40+ years (mean ± SD)
N	32	73	64
Sperm DNA fragmentation (%)	23.94 ± 6.20	28.58 ± 5.70	34.54 ± 7.37
Comparative test	KW= 45.47, P<0.0001 Dunn's multiple comparison test < 30 vs. 30-39, P<0.01 <30 vs 40+, P<0.001 30-39 vs 40+, P<0.001		
Oxidative stress	1.75 ± 0.80	2.01 ± 1.03	2.48 ± 0.89
Comparative test	KW=15.42, P=0.0004, Dunn's multiple comparison test < 30 vs. 30-39, P>0.05 <30 vs 40+, P<0.01 30-39 vs 40+, P<0.01		

The percentage of DNA fragmentation in the sperm of the study group with the increase in age was also

increased by significant statistical significance (KW = 45.47, P <0.0001). With Dunn's multiple comparison test, we gained a distinction of significant statistical significance between AND fragmentation values in male sperm <30 years and those 30-39 years (P <0.01), men <30 years and those 40+ years (P <0.001) and fats 30-39 years and those 40+ years (P <0.001), (Table 2). In the study group males, the increase in the age of oxidative stress also increased with significant statistical significance (KW = 15.42, P = 0.0004). With Dunn's multiple comparison test, we did not distinguish between significant statistical significance between men's <30 years and 30-39 years (P <0.05), but we have distinguished significant statistical significance between values of (<0.01) and between 30-39 years of age and 40+ years of age (P <0.01), (Table 2).

DISCUSSION:

The analysis of the fragmentation of DNA fragmentation has been developed and improved by (Fernández et al., 2003). This method is simple and easy to perform in andrology labs and is available in the market as Halosperm / Halotech kit, DNA, Spain. Our results of fragmented DNA analysis for infertile males taken in the study (p <.0001) are in complete harmony with the results obtained from (Fernández et al., 2003) (p <.001). (Irvine et al., 2000) evaluated the relationship between the parameters of ejaculation and integrity of the fragmented DNA molecule of a group of infertile patients and a group of fertile patients. (Sun et al., 1997) have shown a significant negative correlation between ejaculate parameters and DNA damage to the sperm. Furthermore, in the procedure used with SCD, sperm tails remain intact, allowing sperm cells to be distinguished from other cell types (eg leukocytes). Our results are consistent with studies (Sergerie, et al., 2005) that gained high DNA value fragmented in the infertile male group compared to the male control group. From these results, it can be concluded that the determination of sperm DFI can be used to distinguish infertile males from male fertile (Diallo et al., 2015). Each laboratory technique to analyze DNA fragmented in each androgenic clinic or assisted reproductive technology (ART) should be simple, low cost, and without the need for complex equipment (De Jonge C., 2002). From studies conducted by Sun et al., 1997; Evenson et al., 1999; Spanó et al., 2000, showed that infertile males had higher percentages of fragmented DNA sperm cells than male fertile sperm cells. Moreover, sperm quality is associated with the percentage of fragmented DNA (Irvine et al., 2000; Sakkas et al., 2002). Our results are consistent with the results obtained by Ramzan et al., 2015, which have found the high frequency in significant (p <.001) incidence of fragmented DNA in ejaculates between the infertile group and fertility. Budi Wiweko and Pramety Utami, 2017, have gained significant (p <.001) significant scores of fragmented DNA analysis in infertile male ejaculation compared to male fertility, resulting in results consistent with the results of our study. From our study results, we have gained high frequency (L2-L4) significantly (p <.001) in infertile males compared to male males, resulting in results consistent with the results obtained from (Lu JC et al. , 2010). Our results obtained at significant levels of oxidative stress are consistent with the results obtained by (Badade et al., 2011), which have found the high frequency of oxidative stress in the ejaculate of patients with oligoasthenospermia compared to the control group. The results of our study correlate with the results obtained from (Athayde et al., 2007), which have gained high frequency of oxidative stress at a significant rate (p <.001) in ejaculate samples of patients with leukocytes.

CONCLUSION:

The sperm DFI (Sperm DNA Fragmentation Index) was significant in the infertility patient group compared to the fertile patient group. We can conclude that the results of DNA fragmentation in the sperm are encouraging and can be used for diagnostic purposes in determining male infertility. Such an assessment of the damage to the sperm in the sperm can assist clinicians to help spouses with infertility problems in the selection of IVF / ICSI assisted medical support technique. It is recommended that such an analysis of DNA fragmentation determination in the sperm becomes part of the clinical trials of andrology laboratories. We recommend that an assessment of oxidative stress levels in infertile male ejaculation should be part of routine work in clinical andrology clinics to assist clinicians in determining infertility status and in setting optimal medical treatment.

Competing financial interests:

All authors declare no competing financial interests.

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