International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 11 Issue 12, December 2022

Correlation of Sperm DNA Fragmentation with Age, Semen Parameters and Pregnancy Outcomes

Halida Avdihodzic
Gynecological Center for IVF and Regenerative Medicine, Gynecological Polyclinic "KORAK DO ŽIVOTA", Tuzla, Bosnia and Herzegovina
Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina

Sabina Tinjic
Gynecological Center for IVF and Regenerative Medicine, Gynecological Polyclinic "KORAK DO ŽIVOTA", Tuzla, Bosnia and Herzegovina
Sarajevo Medical School, School of Science and Technology, Sarajevo, Bosnia and Herzegovina

Suada Tinjic
Gynecological Center for IVF and Regenerative Medicine, Gynecological Polyclinic "KORAK DO ŽIVOTA", Tuzla, Bosnia and Herzegovina

***Abstract*— Since 15-20% of married couples worldwide have trouble getting pregnant, infertility has emerged as a major medical problem. Sperm morphological abnormalities, which are frequently related to sperm DNA damage, are one of the reasons of male infertility. However, regular semen examination could not confirm numerous sperm problems, including chromosomal anomalies. The objectives of this study were to first demonstrate the differences in sperm DNA fragmentation among patients of various ages, then to determine whether the characteristics of the semen have an impact on sperm DNA fragmentation, and finally to assess the relationship between sperm DNA fragmentation and pregnancy outcomes using and without the magnetic sell sorting (MACS) method. The study is based on scientific studies that were published between 2000 and 2021 in the databases PubMed, Google Scholar, Scopus, and ScienceDirect, as well as official publications from the World Health Organization (WHO). The study indicates a favourable correlation between age and the sperm DNA fragmentation index (DFI). When comparing SDF with semen parameters, the SDF adversely impacts the sperm mobility. The early embryonic development and pregnancy outcomes were correlated with SDF values. By using MACS method in IVF/ICSI there are showed improvements and better rates in pregnancy outcomes. SDF should be added to the male sample's regular processing, and the MACS method should be used before ART in cases when the DFI value is high.**

Keywords—Sperm DNA fragmentation; semen parameters; pregnancy outcomes; In Vitro Fertilization; MACS

I. INTRODUCTION

Due to infertility 15-20% of married couples over the world have trouble getting pregnant. Male factors account for between 20–70% of cases of infertility, with over 30% of these factors having a direct impact on fertility [1]. In an effort to find the best treatment option for each infertile couple, different infertility treatment techniques have been created and improved over time. Frequently used assisted reproduction technique (ART) for infertility is Intrauterine insemination (IUI) and it is recommended in patients with mild cases of male factor infertility, an ovulation, endometriosis, and unexplained infertility. The majority of the time, severe male factor infertility is treated by in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). ICSI, as opposed to IVF, selects spermatozoa based on morphology and motility, which results in improved male gamete selection. Recent research suggests that even while the sperm appears to be morphologically normal, there is still a chance that there are molecular problems [1, 2].

The initial step in identifying male infertility is traditional semen testing, as described by World Health Organization (WHO) guidelines, which take into account total sperm quantity, concentration, motility, and shape [2]. Sperm DNA fragmentation index (DFI) is used to predict male infertility and has greater diagnostic and prognostic value than standard semen parameters, according to a number of recent research [3, 4]. For male fertility there is defined DNA fragmentation status as > 30% "significant lack of", 15-30% 'reasonable' and < 15% DNA 'high' fertility status. [4]. High levels of sperm DFI are linked to lower rates of fertilization, early embryo development, embryo quality, pregnancy rates, and greater rates of spontaneous miscarriage. Numerous factors, including apoptosis during sperm maturation in the seminiferous tubule epithelium flaws in chromatin packaging and remodeling during the process of spermiogenesis, an increase in reactive oxygen species (ROS), stress, alcoholism, smoking, drug usage, caffeine, poor diet, and old age are additional variables that raise DFI. During sperm maturation, human spermatozoa first migrate through the epididymis from testis with little or no motility. The percentage of motile sperm increases gradually as they pass through the epididymis. Thus, impaired sperm motility may occur due to high levels oxidative stress (OS) caused by overproduced reactive oxygen species (ROS) during sperm maturation process, or a consequence of unbalanced apoptosis [5, 6].

There have been numerous SDF assays established, with the primary techniques being sperm chromatin dispersion (SCD), terminal deoxyuridine nick end labeling (TUNEL), acridine orange test (AOT), sperm chromatin structure assay (SCSA), and aniline blue (AB) staining [7, 8, 9]. TUNEL uses probes to measure single and double DNA strand breaks directly, whereas SCD, SCSA, AOT, and AB stains employ the enhanced acid-induced denaturation susceptibility of damaged sperm DNA. One of these methods is the magnetic-

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 11 Issue 12, December 2022

activated cell-sorting (MACS) method, which uses annexin V-conjugated superparamagnetic microbeads to detect externalized PS residues on apoptotic sperm cells to identify and positively eliminate apoptotic cells from ejaculate [10]. The only way to prevent the selection of spermatozoa that are already scheduled to undergo apoptosis or who are currently going through this process for ICSI is by excluding them using the MACS technique because they can still swim and seem normal. According to certain research, this strategy can decrease the proportion of spermatozoa containing DNA fragments [11, 12], enhance the spermatozoa acrosome reaction [13], enhance the mitochondrial membrane potential [14], and boost the rates of embryo implantation and pregnancy [15]. Before using the sperm for ART operations, this approach also lowers the percentage of sperm with fragmented DNA in the ejaculate [11]. The goal of this study is to demonstrate the relationship between SDF and patient age, semen characteristics, and pregnancy outcomes with and without the MACS method.

II. METHODS

This study is presented as a narrative commentary and is based on scientific articles that were published between 2000 and 2022 in the official publications of the World Health Organization (WHO), as well as in the multiple databases PubMed, Google Scholar, Scopus, and ScienceDirect. The search was limited to human studies published in English and included using the following terms: "sperm DNA fragmentation", "sperm DNA integrity", "sperm DNA damage", "SDF", "DFI", "traditional semen parameters", "conventional semen parameters", "MACS", "pregnancy outcomes." All original research articles, including cohort studies, patient series, randomized and unrandomized controlled trials, were included. From the full-text papers' references, further studies were taken. Additionally, published conference abstracts have been taken into consideration. This study has been reported as per the PRISMA reporting guidelines for Review Articles (Systemic and Narrative review articles).

III. DISCUSSION

A. CORRELATIONF OF DFI AND AGE

Male factor infertility was associated with greater sperm DNA fragmentation, which may play a part in the pathogenesis of male infertility, according to several studies [16, 17, 18]. In both men with normospermia and individuals with aberrant sperm parameters, Das et al. showed a higher DFI in men with greater paternal age [19]. In addition, Belloc et al. discovered that in 1,974 males with normospermia, increasing paternal age was a significant predictor of greater DNA sperm fragmentation [20].

Campos et al found that age has a high significance in patients with altered semen parameters when compared with patients with normal semen parameters (39.50 ± 6.87 vs 37.26 ± 6.76, respectively) [21]. Male aging is linked to sperm DNA fragmentation, altered sperm parameters, and infertility. Age-related ROS build up results in increased oxidative stress, which triggers lipid peroxidation and further ROS production in the mitochondria [22]. Apoptosis or DNA oxidative damage may be brought on by an excess of ROS and a diminished antioxidant capacity as we age.

In a research comprising 215 first-time parents with uncertain reproductive potential, Spano and colleagues [23] found that sperm DNA damage nearly doubled from 25 to 55 years of age. In support of these conclusions, Singh et al. [24] found that males aged 36–57 had a considerably larger percentage of sperm with highly damaged DNA than men aged 20–35 did. They suggested that the increased sperm DNA damage with increasing age may be caused by less effective sperm cell selection procedures as they also detected an age-related decrease in sperm apoptosis.

Older men (>40) exhibited a larger percentage of DFI than younger men and this data have been provided in a number of research [25, 26, 27]. Kaarouch et al. [25] and Alshahrani et al. [26] demonstrated the effect of age on sperm DNA integrity by demonstrating that men over the age of 40 had a much larger percentage of DFI than younger men. Similar findings were made by Vagnini et al. [27], who found a substantial difference in the proportion of sperm with fragmented DNA in patients between the ages of 35 and 40. Furthermore, in two age-dependent groups, 24-34 y and 35-45 y, Plastira et al. [28] evaluated the sperm DNA integrity of men with oligoasthenoteratozoospermia and those with normozoospermia. In comparison to the group of younger men, the older men with oligoasthenoteratozoospermia had a much higher percentage of DFI, according to the researchers. Furthermore, they discovered a strong relationship between sperm cell chromatin degradation and age. The group of males with normozoospermia, however, did not exhibit any variations or connections, according to the scientists. Similar findings were made by Winkle et al. [29]; participants between the ages of 36 and 39 had a considerably lower percentage of DFI than the group of men aged 40 y with abnormal standard semen parameters.

B. CORRELATION OF DFI AND SEMEN CHARACTERISTICS

Up to 80% of infertile men have higher seminal ROS, and numerous studies have demonstrated a link between changed semen parameters and high ROS concentrations. Similarly, the information at hand indicates that oxidative stress plays a significant role in the fragmentation of sperm DNA [30, 31]. As a result, in patients with altered semen parameters their high DFI levels are caused by the elevated ROS concentrations that their poorer-quality semen produces.

These findings are in line with those of other Peruvian studies, such as those of Acosta & Dueas, who discovered that the mean DFI value among patients with altered semen parameters was significantly higher than that among patients with normal semen parameters (22.95 ± 12.25 vs 14.39 ± 9.06); and who discovered that the mean DFI value between patients with altered semen parameters and patients with normal semen parameters differed significantly (21.51 ± 14.18 vs 14.08 ± 7.08) [32, 33]. Other studies employing the SCD test to examine both fertile and infertile patients showed similar findings [34, 35]. On the other hand, the study by Khalili et al. utilizing the acridine orange staining test did not identify any significant variations in the value of DFI between fertile and infertile patients [36]. Due to its low clinical

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 11 Issue 12, December 2022

importance for infertility testing and lack of correlation with other tests, such as SCSA, TUNEL, and SCD, the acridine orange staining assay is dubious and is not advised as a screening test for sperm quality and functional capacity [37].

Acosta et al reported in the study that patients diagnosed as normozoospermic had showed lower significant levels of DFI when compared to oligozoospermic, asthenozoospermic, teratozoospermic and oligoasthenoteratozoospermic patients (26.38±12.94; 23.09±11.45; 17.96±9.23; 22.05±12.15, respectively) [38]. Their data agrees with a number of research [39, 40, 41]. The increased apoptosis of mature spermatozoa may be the cause of this association. Male gamete overproduction is restrained by apoptosis [42]. Apoptosis of mature spermatozoa is linked with elevated ROS levels [43]. A decrease in sperm count could result from DNA damage brought on by oxidative stress accelerating the demise of germ cells [44]. One of the key factors affecting the ability of sperm to fertilize is progressive motility.

When compared to males with oligozoospermia or isolated teratozoospermia, Belloc et al. discovered a significantly higher level of DFI in males with asthenozoospermia alone [45]. Elbashir et al. discovered a strong inverse relationship between DFI and progressive motility in asthenozoospermic men who are infertile and those who have had their fertility confirmed [46]. On the other hand, while having a high median distribution, Varshini et al. observed a statically non-significant difference in DFI using TUNEL between asthenozoospermic patients and normozoospermic patients [39]. The formation of the flagellum during spermatogenesis offers one explanation for the connection between DFI and asthenozoospermia. Cho et al. shown that the development of an aberrant flagellum and poor motility are related to DNA compaction (using protamine or transition protein insufficiency models) [47]. The increased oxidative stress that results in sperm DNA damage and subsequent lipid peroxidation of the sperm membrane, which in turn causes the oxidation of polyunsaturated fatty acids in the plasma membrane and the formation of malondialdehyde (MDA), may be another factor that explains this correlation. MDA causes structural and functional damage to the spermatozoa. High levels of MDA is correlated with high DFI, both having a negative correlation with the progressive motility. Thus, high concentration of ROS can cause decreased sperm motility due to the damage to the axonemal structure or the reduction in intracellular adenosine triphosphate [48, 49, 50]. The decreased sperm motility has also been explained by apoptosis. Oxidative stress causes the generation of spermatozoa with poorly remodelled chromatin. These defective cells have a tendency to enter in an apoptotic pathway associated with motility loss.

There is little knowledge about the pathophysiological process underlying teratozoospermia, despite the fact that it exhibits high phenotypic heterogeneity. The spermatozoa's morphological abnormalities are crucial in defining the male reproductive potential. DNA damage is associated with various aberrant sperm head and flagellum shapes, with the anomaly of the head exhibiting the highest DFI value [51]. Teratozoospermia and DFI have been shown to positively correlate in the past, while other investigations Avendao et al. and Choucair et al. have not shown a significant link between these variables [40, 41, 52, 53, 54]. One of the mechanisms underlying this association is the partial histone replacement by protamine, which results in aberrant chromatin condensation and deforms the sperm's nucleus and overall head shape [55]. An additional explanation might be oxidative damage brought on by aberrant apoptosis. Teratozoospermia may increase due to faulty apoptosis, which may result in the persistence of aberrant spermatozoa that are intended for removal [42]. According to Aydos et al., the relationship between damaged sperm DNA and abnormal sperm morphology may be due to the fact that damaged sperm DNA affects the chromatin structure of the sperm [40].

The findings demonstrated that a high SDF rate was present in 57.14% of patients. It's interesting to note that normozoospermia was diagnosed in 50% of patients with high SDF. 16.6% of patients had lower sperm concentrations, 16.6% had lower sperm motility values, and 16.6% had lower sperm concentration and motility values combined. Patients with both decreased spermatozoon count (oligozoospermia) and impaired sperm motility (asthenozospermia) also had a 95.54% HSDF rate. The findings unmistakably demonstrated that HSDF adversely impacts spermatozoon count and motility in addition to teratozoospermia, which was identified in conjunction with asthenozoospermia or with normozoospermia [56].

C. CORRELATION OF DFI AND PREGNANCY OUTCOMES

Many researchers think SDF has a detrimental effect on embryo quality and pregnancy outcomes after IVF/ICSI [57,48]. High sperm DFI has been linked to lower pregnancy rates in addition to lower fertilization rates and poor embryo quality in IVF, according to research by Zheng et al [59]. The study by Niu et al. found that high DFIs have no effect on oocyte fertilization rates or pregnancy outcomes after IVF, but they do impair embryo quality (rates of good quality embryos and blastocyst development) [60]. The unfavourable impact of high sperm DFI on outcomes of natural pregnancies or IUI pregnancies has generally been acknowledged, despite the fact that researchers' opinions on the association between DFI and IVF/ICSI pregnancy outcomes are contested. Sperm DFIs has been demonstrated to be considerably higher in couples with unexplained infertility [61, 62]. Those with high DFI have noticeably greater rates of pregnancy and early abortions than those with low DFI [63]. Yang et al. found that the early abortion rate in intrauterine insemination (IUI) cycles was significantly increased as sperm DFI increased, while there was no significant difference in ICSI cycles [64].

When SDF results (using the SCD assay) are greater than 20%, IUI pregnancy rates decline in couples with unexplained infertility [65]. When inseminations are performed using samples from males with SDF levels >30%, the likelihood of pregnancy success by IUI is likewise decreased (by 7.0- to 8.7-fold) in the general infertile population (measured by the SCSA in the neat semen. [66, 67, 68].

The majority of IVF/ICSI meta-analyses agree that sperm DNA integrity affects the success of conception. According to research by Li et al., Zini et al., and Zhao et al., higher SDF was linked to lower pregnancy rates with traditional IVF but not with ICSI [69, 70, 71]. However, Osman et al. and Simon et al. demonstrated that increased SDF had a negative impact

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 11 Issue 12, December 2022

on the success of both IVF and ICSI [72, 73]. The latter is now the most comprehensive data compilation. They examined data from 70 research, spanning more than 17,000 IVF/ICSI cycles, and found an association between higher SDF and a decline in clinical pregnancy following IVF or ICSI.

Couples undergoing IVF/ICSI who had greater (vs lower) SDF rates also had a higher chance of miscarriage. These findings were supported by a meta-analysis of 23 IVF/ICSI studies that included 6,771 cycles [74]. This study found that the presence of increased SDF had a deleterious impact on clinical pregnancy rates and miscarriage rates, but not on live birth rates (10 studies; 1,785 couples). A growing body of evidence suggests that live birth rates decline in both IVF and ICSI patients when SDF rates (measured by Comet) exceeded the threshold levels, even though the detrimental effect of SDF on IVF and ICSI cycles has not been reported conclusively [75, 76, 77]. Miscarriage rates that are higher than normal appear to be a regular occurrence in IVF/ICSI cycles performed on increased SDF specimens. Zhao and coworkers (2014) demonstrated that higher SDF had a substantial impact on the chance of miscarriage by combining the data from 14 IVF/ICSI studies with 2,756 couples. These numbers indicate that if loss rates are 10%–15% on average, then couples undergoing IVF/ICSI with spermatozoa from semen specimens with high SDF will experience a miscarriage rate of 23%. If a fertility center performed 1,000 IVF/ICSI cycles year with an average clinical pregnancy rate of 40%, the net effect of SDF would be a reduction in about 80 pregnancies, ultimately leading to a reduction in the live birth rate of up to 15% [71].

The probability of an early miscarriage was found to be modestly enhanced by sperm with immature chromatin above a particular threshold in a novel retrospective study of 1602 pregnancies from IVF and ICSI cycles [78]. In this regard, a prior study found that MACS sorting of semen containing spermatozoa with significant DNA fragmentation decreased the miscarriage rate in ICSI cycles [79]. Additionally, when MACS sperm selection was carried out for 80 infertile couples with an underlying troubling male factor who had ICSI, there was a significantly higher percentage of high-quality embryos and clinical pregnancies compared to the study's control group [80]. Interestingly, no benefit for MACS sperm selection was discovered when large levels of sperm DNA fragmentation were not used as a selection criterion [81].

IV. CONCLUSION

Our research shows that age is positively connected with sperm DNA damage. Furthermore, our findings show that older men are considerably more likely than younger men to have isolated sperm DNA damage. This paper demonstrated that increased sperm DNA damage is related to reduced progressive motility. Additionally, the study lends credence to the notion that sperm DNA fragmentation is more frequently found in males who are infertile. In ICSI trials compared to traditional IVF studies, the extent of the effect size for the negative impact of SDF on IVF and ICSI results appears to be smaller. Overall, the data indicated that MACS sperm selection improved the rates of pregnancy, miscarriage, and live birth. When the MACS sperm-selection approach was used, autologous ICSI cycles demonstrated a substantial and significant improvement in reproductive outcomes, with a large drop in miscarriage rate and a rise in live birth rate. These findings revealed a tight connection between increased miscarriages and high sperm DNA fragmentation levels. Sperm DNA double-strand breaks are the primary source of abortive apoptosis, and this kind of DNA fragmentation is linked to a higher risk of miscarriage. As the MACS procedure is based on the selective elimination of apoptotic cells, the sperm population isolated after this sperm-selection technique presumably had a significant reduction in sperm containing double-strand breaks and thereby reduced the chance that one of these spermatozoa would be selected for ICSI cycles.

Low fertility rates, early embryo development, poor embryo quality, low pregnancy rates, and greater rates of spontaneous miscarriage are all associated with high DFI levels. Because of these factors, numerous researchers have suggested adding sperm DNA fragmentation analysis as a standard and supplemental test in semen analysis. The normal use of the SDF assays is debatable, despite the fact that various tests are available to evaluate SDF, they still lack optimization and precise clinical reference values. As the use of SDF assays in clinical practice is just getting started, future, more thorough research may broaden the application of SDF testing to infertile couples for improved management.

ACKNOWLEDGMENT

There are no acknowledgements associated with this publication.

REFERENCES

[1] L. Candela, L. Boeri, P. Capogrosso, W. Cazzaniga, E. Pozzi, F. Belladelli et al., “Correlation among isolated teratozoospermia, sperm DNA fragmentation and markers of systemic inflammation in primary infertility”, Plos one, 2021; 16 (6).

[2] W. C. Ford, “Comments on the release of the 5th edition of the WHO laboratory manual for the examination and processing of human semen”, Asian J Androl, 2010; 12 (1) , pp. 59-63

[3] M. Bungum, L. Bungum, A. Giwercman, “Sperm chromatin structure assay (SCSA): a tool in diagnosis and treatment of infertility”, Asian J Androl, 2011; 13 (1), pp. 69-75

[4] E. Evgeni, G. Lymberopoulos, S. Touloupidis,B. Asimakopoulos, “Sperm nuclear DNA fragmentation and its association with semen quality in Greek men”, Andrologia, 2015; 47 (10) , pp. 1166-1174

[5] S. Bisht, M. Faiq, M. Tolahunase, R. Dada, “Oxidative stress and male infertility”, Nat. Rev. Urol., 2017; 14 (8) pp. 470-485

[6] K. K. Shukla, A. A. Mahdi, S. Rajender, “Apoptosis, spermatogenesis and male infertility”, Front Biosci. (Elite Ed)., 2012; 1 (4) pp. 746-754

[7] S. M. Bhattacharya, “Association of Various Sperm Parameters With Unexplained Repeated Early Pregnancy Loss–Which Is Most Important?”, Int Urol Nephrol, 2008; 40(2):391–5.

[8] M. L. Eisenberg, K. J. Sapra, S. D. Kim, Z. Chen, G. M. Buck Louis, “Semen Quality and Pregnancy Loss in a Contemporary Cohort of Couples Recruited Before Conception: Data From the Longitudinal Investigation of Fertility and the Environment (LIFE) Study”, Fertil Steril, 2017; 108(4):613–9.

[9] X. B. Zhu, Q. Chen, W. M. Fan, Z. H. Niu, B. F. Xu, A. J. Zhang, “Sperm DNA Fragmentation in Chinese Couples With Unexplained Recurrent Pregnancy Loss”, Asian J Androl, 2019; 22(3):296–301.

[10] T. H. Lee, C.H. Liu, Y. T. Shih, H. M. Tsao, C. C. Huang, H. H. Chen et al., “Magnetic-activated cell sorting for sperm preparation reduces spermatozoa with apoptotic markers and improves the acrosome reaction in couples with unexplained infertility”, Hum. Reprod., 2010; 25, 839–846

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 11 Issue 12, December 2022

[11] V. Y. Rawe, H. U. Boudri, S. C. Alvarez, M. Carro, S. Papier, F. Nodar, "Healthy baby born after reduction of sperm DNA fragmentation using cell sorting before ICSI", Reprod BioMed Online., 2010; 20: 320–3.
[12] S. Bucar, A. Gonçalves, E. Rocha, A. Barros, M. Sousa, R. Sá, "DNA fragmentation in human sperm after magnetic-activated cell sorting", J Assist Reprod Genet., 2015; 32:147–54.
[13] S. Grunewald, T. Baumann, U. Paasch, H. J. Glander, "Capacitation and acrosome reaction in nonapoptotic human spermatozoa", Ann N Y Acad Sci., 2006; 1090:138–46.
[14] C. de Vantéry Arrighi, H. Lucas, D. Chardonnens, A. de Agostini, "Removal of spermatozoa with externalized phosphatidylserine from sperm preparation in human assisted medical procreation: effects on viability, motility and mitochondrial membrane potential", Reprod Biol Endocrinol., 2009; 7:1.
[15] E. K. Dirican, O. D. Ozgün, S. Akarsu, K. O. Akin, O. Ercan, M. Uğurlu et al., "Clinical outcome of magnetic activated cell sorting of nonapoptotic spermatozoa before density gradient centrifugation for assisted reproduction", J Assist Reprod Genet., 2008; 25:375–81.
[16] A. Zini, M. A. Fischer, S. Sharir, B. Shayegan, D. Phang, K. Jarvi, "Prevalence of abnormal sperm DNA denaturation in fertile and infertile men", Urology, 2002; 60, pp. 1069-1072
[17] S. I. Moskovtsev, J. Willis, J. White, J. B.Mullen, "Sperm DNA damage: correlation to severity of semen abnormalities", Urology, 2009; 74, pp. 789-793
[18] Q. Chen, J. Z. Zhao, X. Xue, G. X. Zhu, "The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis", Reprod Toxicol, 2019; 82, pp. 50-55
[19] M. Das, N. Al-Hathal, M. San-Gabriel, S. Phillips, I. J. Kadoch, F. Bissonnette et al., "High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age", J Assist Reprod Genet, 2013; 30, pp. 843-848
[20] S. Belloc, M. Benkhalifa, M. Cohen-Bacrie, A. Dalleac, E. Amar, A. Zini, "Sperm deoxyribonucleic acid damage in normozoospermic men is related to age and sperm progressive motility", Fertil Steril, 2014; 101, pp. 1588-1593
[21] L. G. A. Campos, L. C. Requejo, C. A. R. Minano, J. D. Orrego, E. C. Loyaga, L. Gonzales Cornejo, "Correlation between sperm DNA fragmentation index and semen parameters in 418 men seen at a fertility center", JBRA Assisted Reproduction 2021;25(3):349-357
[22] S. Gunes, G. N. Hekim, M. A. Arslan, R. Asci, "Effects of aging on the male reproductive system", J Assist Reprod Genet. 2016;33:441-54.
[23] M. Spano, J. P. Bonde, H. I. Hjollund, H. A. Kolstad, E. Cordelli, G. Leter, "Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team", Fertil Steril. 2000;73: 43–50. 49.
[24] N. P. Singh, C. H. Muller, R. E. Berger, "Effects of age on DNA doublestrand breaks and apoptosis in human sperm", Fertil Steril. 2003;80: 1420–30.
[25] I. Kaarouch, N. Bouamoud, A. Madkour, N. Louanjli, B. Saadani, S. Assou et al., "Paternal age: negative impact on sperm genome decays and IVF outcomes after 40 years", Mol Reprod Dev. 2018; 85:271–80.
[26] S. Alshahrani, A. Agarwal, M. Assidi, A. M. Abuzenadah, D. Durairajanayagam, A. Ayaz et al., "Infertile men older than 40 years are at higher risk of sperm DNA damage", Reprod Biol Endocrinol. 2014; 12:103.
[27] L. Vagnini, R. L. Baruffi, A. L. Mauri, C. G. Petersen, F. C. Massaro, A. Pontes A et al., "The effects of male age on sperm DNA damage in an infertile population", Reprod Biomed Online. 2007; 15:514–19.
[28] K. Plastira, P. Msaouel, R. Angelopoulou, K. Zanioti, A. Plastiras, A. Pothos et al., "The effects of age on DNA fragmentation, chromatin packaging and conventional semen parameters in spermatozoa of oligoasthenoteratozoospermic patients", J Assist Reprod Genet. 2007; 24:437–43.
[29] T. Winkle, B. Rosenbusch, F. Gagsteiger, T. Paiss, N. Zoller, "The correlation between male age, sperm quality and sperm DNA fragmentation in 320 men attending a fertility center", J Assist Reprod Genet. 2009; 26:41–46.
[30] A. Majzoub, M. Arafa, W. El Ansari, M. Mahdi1, A. Agarwal, S. Al-Said et al., "Correlation of oxidation reduction potential and total motile sperm count: its utility in the evaluation of male fertility potential", Asian J Androl. 2019;22:317-22.
[31] V. M. Iommiello, E. Albani, A. Di Rosa, A. Marras, F. Menduni, G. Morreale et al., "Ejaculate oxidative stress is related with sperm DNA fragmentation and round cells", Int J Endocrinol. 2015;2015:321901.
[32] L. G. Acosta, J. C. Dueñas J C, "Correlation between semen parameters and sperm DNA fragmentation in men attending a fertility center", Rev Iberoam Fert Rep Hum. 2014;31:44- 52.
[33] L. G. Acosta, C. Rivas, L. Chiscul, J. Díaz, "Correlation between semen parameters, sperm DNA fragmentation and oxidative stress in patients attending in a fertility center in northern Perú", Rev Iberoam Fert Rep Hum. 2015;32:24-34.
[34] E. Evgeni, G. Lymberopoulos, M. Gazouli, B. Asimakopoulos, "Conventional semen parameters and DNA fragmentation in relation to fertility status in a Greek population", Eur J Obstet Gynecol Reprod Biol. 2015;188:17-23.
[35] B. Wiweko, P. Utami, "Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility", Basic Clin Androl. 2017;27:1.
[36] M. A. Khalili, F. Aghaie-Maybodi, M. Anvari, A. R. Talebi, "Sperm nuclear DNA in ejaculates of fertile and infertile men: correlation with semen parameters", Urol J Tehran. 2006;3:154-9.
[37] K. R. Chohan, T. Jeanine, J. T. Griffin, M. Lafromboise, C. J. De Jonge, D. T. Carrel, "Comparison of chromatin assays for DNA fragmentation evaluation in human sperm", J Androl. 2006;27:53-9.
[38] L.G. A. Campos, L. C. Requejo, C. A. R. Miñano, J. D. Orrego, E. C. Loyaga, L. G. Cornejo, "Correlation between sperm DNA fragmentation index and semen parameters in 418 men seen at a fertility center", JBRA Assisted Reproduction 2021;25(3):349-357
[39] J. Varshini, B. S. Srinag, G. Kalthur, H. Krishnamurthy, P. Kumar, S. B. S. Rao, S. K. Adiga S K, "Poor sperm quality and advancing age are associated with increased sperm DNA damage in infertile men", Andrologia. 2011;44:642-9.
[40] O. S. Aydos, Y. Yükselten, F. Kaplan, A. Sunguroğlu, K. Aydos, "Analysis of the correlation between sperm DNA integrity and conventional semen parameters in infertile men", Turk J Urol. 2015;41:191-7.
[41] F. B. Choucair, E. G. Rachkidi, G. C. Raad, E. M. Saliba, N. S. Zeidan, R. A. Jounblat et al., "High level of DNA fragmentation in sperm of Lebanese infertile men using Sperm Chromatin Dispersion test", Middle East Fertil Soc J. 2016;21:269-76
[42] D. Sakkas, E. Mariethoz, G. Manicardi, D. Bizzaro, P. G. Bianchi, U. Bianchi, "Origin of DNA damage in ejaculated human spermatozoa", Rev Reprod. 1999;4:31-7.
[43] A. Agarwal, L. H. Sekhon, "Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: it is justified?", Indian J Urol 2011; 27:74-85.
[44] A. Agarwal, R. A. Saleh, M. A. Bedaiwy, "Role of reactive oxygen species in the pathophysiology of human reproduction", Fertil Steril. 2003;79:829-43.
[45] S. Belloc, M. Benkhalifa, M. Cohen-Bacrie, A. Dalleac, H. Chahine, E. Amar et al., "Which isolated sperm abnormality is most related to sperm DNA damage in men presenting for infertility evaluation", J Assist Reprod Genet. 2014;31:527- 32.
[46] S. Elbashir, Y. Magdi, A. Rashed, M. A. Ibrahim, Y. Edris, A. M. Abdelaziz, "Relationship between sperm progressive motility and DNA integrity in fertile and infertile men", Middle East Fertil Soc J2018;23:195-8
[47] C. Cho, W. D. Willis, E. H. Goulding, H. Jung-Ha, Y. C. Choi, N. B. Hecht et al., "Haploinsufficiency of protamine-1 or -2 causes infertility in mice", Nat Genet. 2001;28:82-6.
[48] A. Agarwal, R. K. Sharma, R. Sharma, M. Assidi, A. M. Abuzenadah, S. Alshahrani et al., "Characterizing semen parameters and their association with reactive oxygen species in infertile men", Reprod Biol Endocrinol. 2014;12:33.
[49] M. Dorostghoal, S. R. Kazeminejad, N. Shahbazian, M. Pourmehdi, A. Jabbari, "Oxidative stress status and sperm DNA fragmentation in fertile and infertile men", Andrologia. 2017;49:e12762.
[50] S. Tsunoda, N. Kawano, K. Miyado, N. Kimura, J. Fujii, "Impaired fertilizing ability of superoxide dismutase 1-deficient mouse sperm during in vitro fertilization", Biol Reprod.2012;87:121.
[51] R. J. Aitken, G. N. De luliis, "On the possible origins of DNA damage in human spermatozoa", Mol Hum Reprod. 2010;16:3- 13.
[52] N. Eskandari, M. Tavalaee, D. Zohrabi, M. Nasr-Esfahani, "Association between total globozoospermia and sperm chromatin defects", Andrologia. 2017;50:e12843.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 11 Issue 12, December 2022

[53] J. García-Ferreyra, L. Villegas, R. Romero, P. Zavala, R. Hilario, G. Casafranca et al., "Sperm DNA fragmentation is significantly increased in those men with morphologically abnormal spermatozoa", JFIV Reprod Med Genet. 2014;2:131.
[54] C. Avendaño, A. Franchi, S. Taylor, M. Morshedi, S. Bocca, S. Oehninger, "Fragmentation of DNA in morphologically normal human spermatozoa", Fertil Steril. 2009;91:1077-84.
[55] Y. Ma, N. Xie, Y. Li, B. Zhang, D. Xie, W. Zhang et al., "Teratozoospermia with amorphous sperm head associate with abnormal chromatin condensation in a Chinese family", Syst Biol Reprod Med. 2019;65:61
[56] S. Tinjić, M. Ibrišimović, "Introduction Of A Routine Sperm DNA Fragmentation Test As A Significant Help In Evaluating Male Fertility Potential", Asian Journal of Science and Technology, 2019; 10: 10407-410
[57] M. R. Virro, K. L. Larson-Cook, D. P. Evenson, "Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles", Fertil Steril 2004;81:1289-95. 24.
[58] T. Bounartzi, K. Dafopoulos, G. Anifandis, C. Messini, C. Koutsonikou, S. Kouris et al., "Pregnancy prediction by free sperm DNA and sperm DNA fragmentation in semen specimens of IVF/ICSI-ET patients", Hum Fertil (Camb) 2016;19:56-62.
[59] W. W. Zheng, G. Song, Q. L. Wang, S. W. Liu, X. L. Zhu, S. M. Deng et al., "Sperm DNA damage has a negative effect on early embryonic development following in vitro fertilization", Asian J Androl 2018;20:75-9.
[60] Z. H. Niu, H. J. Shi, H. Q. Zhang, A. J. Zhang, Y. J. Sun, Y. Feng, "Sperm chromatin structure assay results after swim-up are related only to embryo quality but not to fertilization and pregnancy rates following IVF", Asian J Androl 2011;13:862-6.
[61] K. Oleszczuk, L. Augustinsson, N. Bayat, A. Giwercman, M. Bungum, "Prevalence of high DNA fragmentation index in male partners of unexplained infertile couples", Andrology 2013;1:357-60.
[62] X. B. Zhu, Q. Chen, W. M. Fan, Z. H. Niu, B. F. Xu, A. J. Zhang, "Sperm DNA fragmentation in Chinese couples with unexplained recurrent pregnancy loss", Asian J Androl. 2019;21:1–6.
[63] F. W. Vandekerckhove, I. De Croo, J. Gerris, E. V. Abbeel, P. De Sutter, "Sperm chromatin dispersion test before sperm preparation is predictive of clinical pregnancy in cases of unexplained infertility treated with intrauterine insemination and induction with clomiphene citrate", Front Med (Lausanne) 2016;3:63.
[64] H. Yang, G. Li, H. Jin, Y. Guo, Y. Sun, "The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle", Transl Androl Urol. 2019;8(4):356–65.
[65] E. Jerre, M. Bungum, D. Evenson, A. Giwercman, "Sperm chromatin structure assay high DNA stainability sperm as a marker of early miscarriage after intracytoplasmic sperm injection", Fertil. Steril. 2019, 112, 46–53
[66] P. Sánchez-Martín, M. Dorado-Silva, F. Sánchez-Martín, M. Martínez, S. D. Johnston, J. Gosálve, "Magnetic cell sorting of semen containing spermatozoa with high DNA fragmentation in ICSI cycles decreases miscarriage rate", Reprod. Biomed. Online 2017, 34, 506–512.
[67] N. Ziarati, M. Tavalaee, M. Bahadorani, M. H. Nasr-Esfahani, "Clinical outcomes of magnetic activated sperm sorting in infertile men candidate for ICSI", Hum. Fertil. 2018, 22, 118–125.
[68] L. Romany, N. Garrido, Y. Motato, B. Aparicio, J. Remohí, M. Meseguer, "Removal of annexin V–positive sperm cells for intracytoplasmic sperm injection in ovum donation cycles does not improve reproductive outcome: A controlled and randomized trial in unselected males", Fertil. Steril. 2014, 102, 1567–1575.
[69] Z. Li, L. Wang, J. Cai, H. Huang, "Correlation of sperm DNA damage with IVF and ICSI outcomes: A systematic review and meta-analysis", Journal of Assisted Reproduction and Genetics 2006; 23, 367– 376.
[70] A. Zini, W. Jamal, L. Cowan, N. Al-Hathal, "Is sperm DNA damage associated with IVF embryo quality? A systematic review", Journal of Assisted Reproduction and Genetics 2011; 28, 391– 397.
[71] J. Zhao, Q. Zhang, Y. Wang, Y. Li, "Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: A systematic review and meta-analysis", Fertility and Sterility 2014; 102, 998– 1005.
[72] A. Osman, H. Alsomait, S. Seshadri, T. El-Toukhy, Y. Khalaf, "The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: A systematic review and meta-analysis", Reprod Biomed Online 2015; 30, 120– 127.
[73] L. Simon, B. R. Emery, D. T. Carrell, "Review: Diagnosis and impact of sperm DNA alterations in assisted reproduction", Best Practice & Research Clinical Obstetrics & Gynaecology 2017; 44, 38– 56.
[74] C. Deng, T. Li, Y. Xie, Y. Guo, Q. Y. Yang, X. Liang et al., "Sperm DNA fragmentation index influences assisted reproductive technology outcome: A systematic review and meta-analysis combined with a retrospective cohort study", Andrologia, 2015; 51.
[75] M. Cissen, M. V. Wely, I. Scholten, S. Mansell, J. P. Bruin, B. W. Mol et al., "Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: A systematic review and meta-Analysis", PLoS One, 2016; 11.
[76] J. A. Collins, K. T. Barnhart, P. N. Schlegel, "Do sperm DNA integrity tests predict pregnancy with in vitro fertilization?", Fertility and Sterility, 2008; 89, 823– 831
[77] J. Nicopoullos, A. Vicens-Morton, S. E. M. Lewis, K. Lee, P. Larsen, J. Ramsay 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, 1915– 1923
[78] E. Jerre, M. Bungum, D. Evenson, A. Giwercman, "Sperm chromatin structure assay high DNA stainability sperm as a marker of early miscarriage after intracytoplasmic sperm injection", Fertil. Steril. 2019, 112, 46–53.
[79] P. Sánchez-Martín, M. Dorado-Silva, F. Sánchez-Martín, M. Martínez, S. D. Johnston, J. Gosálvez, "Magnetic cell sorting of semen containing spermatozoa with high DNA fragmentation in ICSI cycles decreases miscarriage rate", Reprod. Biomed. Online 2017, 34, 506–512.
[80] N. Ziarati, M. Tavalaee, M. Bahadorani, M. H. Nasr-Esfahani, "Clinical outcomes of magnetic activated sperm sorting in infertile men candidate for ICSI", Hum. Fertil. 2018, 22, 118–125.
[81] L. Romany, N. Garrido, Y. Motato, B. Aparicio, J. Remohí, M. Meseguer, "Removal of annexin V–positive sperm cells for intracytoplasmic sperm injection in ovum donation cycles does not improve reproductive outcome: A controlled and randomized trial in unselected males", Fertil. Steril. 2014, 102, 1567–1575