

The Effect of Recombinant Human Follicle-Stimulating Hormone on Sperm DNA Fragmentation and Sperm Parameters in Oligozoospermic Infertile Men



Atefeh Verdi^{1*}, Seyedeh Saeideh Sahraei¹, Elham Asa¹, Rahil Jannatifar¹, Mohamad Bagher Masaeimanesh¹

1. Department of Reproductive Biology, Qom Branch, Academic Center for Education, Culture, and Research (ACECR), Qom, Iran.



Citation Verdi A, Sahraei SS, Asa E, Jannatifar R, Masaeimanesh MB. The Effect of Recombinant Human Follicle-Stimulating Hormone on Sperm DNA Fragmentation and Sperm Parameters in Oligozoospermic Infertile Men. *Research in Molecular Medicine*. 2020; 8(2):55-62. <https://doi.org/10.32598/rmm.8.2.1130.1>

 <https://doi.org/10.32598/rmm.8.2.1130.1>



Article Type:

Research Article

Article info:

Received: 8 Feb 2020

Revised: 10 Mar 2020

Accepted: 5 Apr 2020

Keywords:

DNA fragmentation, Malondialdehyde, Oxidative stress, Oligozoospermia, Recombinant human follicle-stimulating hormone

ABSTRACT

Background: One of the main causes of male infertility is the negative effects of oxidative stress. Follicle-Stimulating Hormone (FSH) plays an essential role in spermatogenesis, as well as in the maintenance of sperm DNA integrity. This study aimed to determine whether the recombinant human Follicle-Stimulating Hormone (rhFSH) treatment of sperm parameters could positively affect sperm DNA and oxidative DNA fragmentation in oligozoospermic infertile men.

Materials and Methods: This interventional study was carried out on a sample of 50 oligozoospermic infertile men. To this end, sperm DNA fragmentation and ROS, as an oxidative stress marker, were measured before and after treatment with rhFSH sperm parameters.

Results: The sperm parameters (concentration, mobility, and morphology) were significantly different in the oligozoospermic infertile patients before and after the rhFSH treatment ($P < 0.05$). Moreover, sperm DNA fragmentation had a significant decrease in patients after the FSH treatment ($P < 0.05$). Besides, the ROS level in sperm and the malondialdehyde level of seminal plasma significantly decreased after the treatment ($P < 0.05$).

Conclusion: Treatment with rhFSH significantly improved sperm parameters. Furthermore, the treatment led to a significant reduction of sperm DNA fragmentation and oxidative stress in the oligozoospermic infertile patients. Similarly, the malondialdehyde concentration markedly decreased in correlation with DNA fragmentation after the rhFSH treatment.

Introduction

Infertility is one of the most critical problems that can be caused by lifestyle changes and environmental stress. According to studies, environmental factors such as pesticides, exogenous estrogen, heavy metals, and free radicals can harm

spermatogenesis and reduce the number of sperms in men. One of these factors that affects fertility in men is Reactive Oxygen Species (ROS) [1]. Oxidative stress is generally used to show whether the amount of antioxidants is either high or low in the cell. Various physiological processes produce ROS. Low production of ROS plays an essential role in the sperm performance (DNA compression and its capacity). However, ROS can break

* Corresponding Author:

Atefeh Verdi, PhD. Candidate.

Address: Department of Reproductive Biology, Qom Branch, Academic Center for Education, Culture, and Research (ACECR), Qom, Iran.

Phone: +98 (912) 8528559

E-mail: atefeh.verdi@yahoo.com

down DNA and increase the risk of a malignant testicular tumor when produced in high levels. Sperm cells are particularly susceptible to the damage induced by excessive ROS because, on the one hand, their plasma membranes are full of polyunsaturated fatty acids, and, on the other hand, cytoplasmic antioxidant enzymes are deficient in sperms [1].

ROS induces lipid peroxidation in the sperm membrane, and peroxidation of the resulting fatty acids has toxic effects on sperms and decreases sperm function [2]. Lipid peroxidation leads to the development of several products, Most Notably Malondialdehyde (MDA) and isoprostane F_{2α} [3-5]. The most commonly used biomarker for lipid peroxidation in sperms is MDA [4]. Fraczek et al. showed that the level of seminal plasma MDA was higher than that of normospermic situation in pathologic conditions, including asthenospermia [5]. The sperm DNA Fragmentation Index (DFI) in infertile men is associated with a defect in spermatogenesis [6]. DFI in mature spermatozoa can be due to defects in chromatin packaging resulting from endogenous fractures in DNA or the apoptosis process before sperm ejaculation. High levels of ROS production can lead to DNA damage. Environmental factors such as age, drug use, and cigarette smoking, hormonal factors, and increased testicular temperature are also other causes of sperm DNA fragmentation [7, 8]. According to previous studies, the use of Follicle-Stimulating Hormone (FSH) can increase sperm concentration and the number of spermatogonia.

Moreover, under this treatment, the pregnancy rate increases in oligozoospermic infertile men with an average level of gonadotropin plasma [9]. Some researchers showed that after the FSH treatment, sperm quality increased significantly [10, 11]. Furthermore, in infertile men with unknown reasons, a positive effect was observed on the concentration and accumulation of sperm DNA after the FSH treatment [12]. Therefore, to obtain background information as well as to predict the success rate of the FSH treatment and its results, it is essential to accurately evaluate male infertility.

According to the above-mentioned statements, our study aimed to assess sperm parameters, as well as DFI, ROS, and MDA levels, in oligozoospermic infertile men with low FSH level after the FSH treatment. On this basis, one can assume that the FSH treatment with rhFSH can function as an anti-apoptosis factor during the general process of spermatogenesis and result in the release of highly fertile sperm cells with the lowest amount of DNA fragmentation.

Materials and Methods

Study design

A semen sample will be taken from 50 infertile men that referred to highly specialized infertility treatment center of ACECR (academic center of education, culture, and research, Qom Branch). The inclusion criteria were the low level of FSH (<1.7 mIU/mL), aged 25–45 years, history of infertility for at least 2 years, sperm concentration <15×10⁶ (oligozoospermia) according to 2010 World Health Organization criteria [13, 14]. Under the supervision of a urologist, all enrolled patients received subcutaneous recombinant FSH (Gonal-f) treatment, 75 IU every other day, starting from Visit 1, for three months, three times a week. Their semen and blood samples were collected before and after FSH treatment and kept in sterile containers to be used in various tests. Then, sperm parameters, sperm DNA fragmentation, and ROS, as a marker of oxidative stress, were measured before and after treatment with rhFSH.

Semen sample analysis

Semen samples were obtained by masturbation after 3–5 days of sexual abstinence and stored in sterile containers. The samples were allowed to liquefy for 30 min and were examined for seminal parameters according to WHO criteria (WHO, 2010). Semen parameters, including motility, morphology, and concentration were assessed by light microscopy, Papanicolaou staining, and Neubauer chamber, respectively.

Hormonal analysis

After the collection of blood samples, they were immediately centrifuged for 10 min at 3000 rpm (Hettich, EBA20, UK) and serum samples were stored at -70°C. We used commercial kits for the assessment of hormonal profile (FSH; mIU/mL, Cat.N.DE1288).

Assessment of DNA fragmentation

For the assessment of DNA fragmentation, we purchased the Halosperm® kit, INDAS laboratories. Sperm samples were used for the SCD test, which was carried out according to the manufacturer's instructions. Slides were stained with Wrights stain (Merck 1.01383.0500). For each sample, a minimum of 500 sperm were evaluated under the ×100 light field microscopy viewing. Sperms without fragmented DNA exhibit large or medium halos, whereas sperms with fragmented DNA appear with small

halos, no halos, and solidly stained nuclei, or no halos and irregular or faintly stained nuclei (degraded) [15].

ROS analysis

2',7'-Dichlorofluorescein Diacetate (DCFH-DA; Sigma Chemical Co., Germany), a specific probe for detection of intracellular H₂O₂, is a cell-permeable stain. DCFH is oxidized selectively by the free intracellular H₂O₂ into DCF that binds to DNA and emits green fluorescence. Briefly, we prepared a 2.5 mM stock solution of H₂D-CFDA in Dimethyl Sulfoxide (DMSO, Merck, Darmstadt, Germany) which was stored at 70. To measure the amount of ROS, we add DCFH-DA (5 mM) to the 1 mL sperm suspension and incubate it at 25 for 40 minutes in dark. Aliquots were subsequently analyzed using a flow cytometer. Green Fluorescence (DCF) was evaluated between 500 and 530 nm [16].

Assessment of lipid peroxidation

Seminal Malondialdehyde (MDA) is assumed as a direct measure of lipid peroxidation and detected by ELISA Kit (Zell Bio GmbH, Wurttemberg, Germany) at a detection range of 0.125–2 mM (125–2000 mmol/L).

Statistical analysis

Semen parameters were analyzed before and after treatment with SPSS V. 20. The paired sample t test was performed using Tukey's complement test to examine DNA damage before and after treatment. Our hypothesis has two domains. The significant level for the P value was less than 0.05. The Spearman test examined the correlation between MDA and sperm parameters and the fragmentation of DNA.

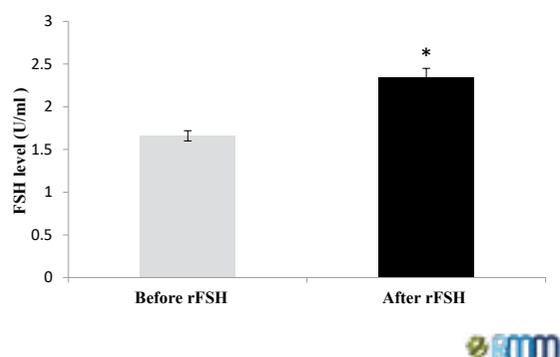


Figure 1. Comparison of FSH level analysis before and after the treatment with rhFSH. FSH level in oligozoospermic infertile men after three months of treatment showed a significant increase compared to what was before the treatment. *P<0.05.

Results

In this study, sperm parameters and hormonal profile, as well as DFI, ROS, and MDA levels were assessed before and after the FSH treatment. The participating oligozoospermic infertile patients were not significantly different in terms of baseline clinical and hormonal characteristics. All patients had a DFI average of below 30% at baseline and a low plasma blood FSH level of below 1.7 mIU/mL. The rhFSH treatment was followed properly by all the participants for three months, as provided in the study protocol.

Figure 1 shows the FSH hormone test in the plasma of the patients before and after the rhFSH treatment. The FSH hormone level was initially low (1.66±0.06 mIU/mL) in the oligozoospermic infertile patients, which significantly increased (2.34 ± 0.11 mIU/mL) three months after the treatment (P<0.05).

Table 1 presents a comparison of the sperm parameters. In the oligozoospermic infertile patients with a low FSH level, a significant improvement was observed in the sperm concentration (8.13±1.80 ml vs. 13.06±2.51106/ml), total sperm motility (30.42±0.60 % vs. 33.18±1.21 %) and morphology three months after the rhFSH treatment (P<0.05).

DFI change from the baseline was investigated for all the patients. The results of the DFI average values in Figure 2 showed that in the oligozoospermic infertile patients with a low FSH level and high sperm DNA fragmentation (33.9±6.58%), sperm DNA fragmentation showed a significant decrease (22.64±6.3 %) three months after the rhFSH treatment (P<0.05).

Regarding the ROS, its level had a significant decrease in spermatozoa in the patients after the treat-

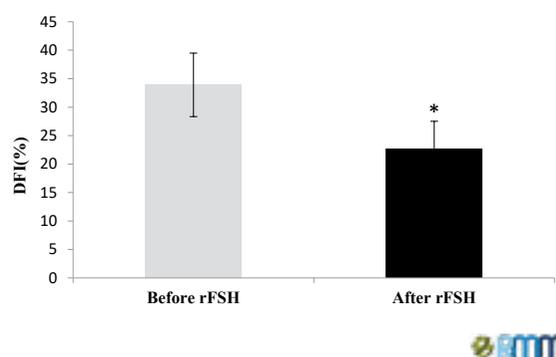


Figure 2. Comparison of sperm DNA fragmentation before and after the treatment with rhFSH. The percentage of DNA fragmentation in oligozoospermia infertile men significantly decreased after three months of rhFSH treatment compared to what was before the treatment. *P<0.05.

Table 1. Comparison of sperm parameters and hormonal analysis before and after the treatment with rhFSH

Sperm Parameters	Before The Treatment (n=50)	After The Treatment (n=50)
Volume (mL)	3.58±0.12	3.52±0.18
Sperm concentration (10 ⁶ /mL)	8.13±1.80	13.06±2.51*
Total motility	30.42±0.60	33.18 ± 1.21*
Progressive motility (a+b)	20.48±2.57	42.523.27*
Non-progressive motility	43.24±2.30	36.001.19*
Immotile sperm	29.811.63	19.520.572*
Abnormal morphology	99.12±0.11	97.02±0.16*

Data are presented as Mean±SD

* P<0.05

ment (P<0.05). Figure 3 shows that the ROS level was 34.55±2.89 % before the treatment, which reduced to 26.23±3.68 % after the treatment.

Our results showed that the MDA concentration significantly declined as a specific lipid peroxidation marker (2.99±0.15 vs. 2.13±0.08 ng/ml; P=0.05) (Figure 4) and that the seminal plasma MDA level of oligozoospermia was directly correlated with DNA fragmentation ($r=+0.228$; P=0.01) (Figure 5).

Discussion

Many research studies have confirmed the effect of FSH on infertility. However, no studies have been performed on FSH treatment in oligospermia patients and its effect on oxidative stress and malondialdehyde levels and their relationship. This study is the first study to evaluate sperm DNA fragmentation and oxidative stress after treatment with rhFSH in oligozoospermic infertile men.

In the present study, the effects of rhFSH were examined on semen parameters, ROS and MDA levels, and sperm DNA fragmentation in infertile men. According to the findings, the administration of rhFSH at a dose of 75 IU, three times a week for three months, significantly improved the sperm parameters (concentration, number, motility, and morphology). Moreover, the treatment helped to markedly reduce ROS and MDA levels as well as sperm DNA fragmentation of spermatozoa in infertile men with oligozoospermia. Since previous studies found a high ROS level in semen samples of 25% to 42% of infertile individuals, ROS is regarded as a crucial factor in sperm function impairment. Moreover, ROS is involved in many physiological activities of spermatozoa, and thus its overexpression causes sperm DNA damage and consequently activates the apoptosis pathway. The degree of oxidative stress injury depends on not only the amount of ROS but also the duration of exposure to external factors such as oxygen pressure, temperature, and the like [8, 16]. In the sperm DNA structure, free radicals

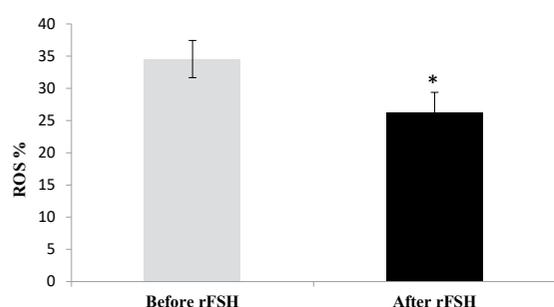


Figure 3. Comparison of ROS level before and after treatment with rhFSH. The percentage of ROS level in oligozoospermic infertile men after three months of treatment significantly decreased compared to what was before the treatment. *P<0.05.

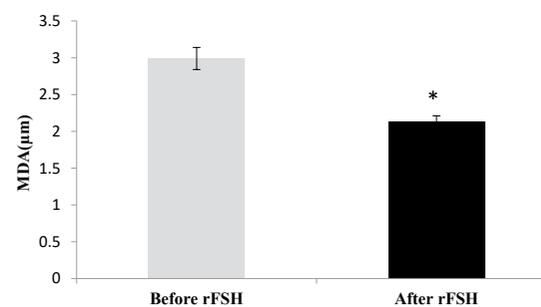


Figure 4. Comparison of seminal malondialdehyde (MDA) before and after treatment with rhFSH. The mean seminal MDA value decreased significantly after treatment with rhFSH compared to what was before the treatment. Statistical analysis was performed by the paired t test. * P<0.05

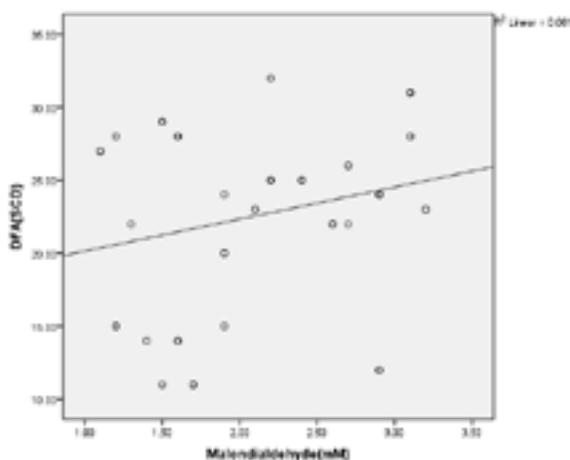


Figure 5. Correlation between sperm DNA fragmentation and MDA. After rhFSH treatment, DNA fragmentation correlated positively with MDA ($r=0.445$, $P=0.05$).

can oxidize purine and pyrimidine bases, break one or two strands, and delete nucleotides, leading to the creation of frameshift mutation and changes in deoxyribose. These changes in sperm DNA can lead to infertility [17]. This study demonstrated that the ROS level in oligozoospermia significantly decreased after the 3-month rhFSH treatment. Hormone therapy is a treatment for infertile oligozoospermia patients [9].

In the present study, oligozoospermia patients with a high DFI level were treated with rhFSH, leading to a significant reduction of the DFI level. On this basis, one can assume that the FSH treatment with rhFSH can function as an anti-apoptosis factor during the general process of spermatogenesis. This intervention will result in the release of highly fertile sperm cells with the lowest amount of DNA fragmentation. The findings showed that FSH plays an essential role in spermatogenesis, especially in the maintenance of sperm DNA integrity. According to the obtained results, the recombinant FSH therapy can be a routine and experimental clinical treatment, which increases sperm concentration and spermatogonia in people with low semen concentration, but with normal plasma gonadotropin. This treatment is also proven to be capable of improving fertility outcomes of Assisted Reproductive Technique (ART) in oligozoospermia [3, 10, 11, 13, 18-20]. In line with the studies mentioned above, in this study, the DFI level of >30% significantly decreased in the oligozoospermia patients treated with rhFSH (75 IU) for three months.

Decreased seminal plasma quality can be one of the factors that increases infertility and causes successive failures in infertility treatments. Decreased seminal

plasma also results in decreased antioxidant capacity, decreased ROS level, and increased sperm DNA fragmentation [21, 22]. Similarly, this study also showed that the seminal plasma level significantly increased after the rhFSH treatment in oligozoospermia. Some researchers reported significant improvements in sperm parameters and sperm nucleus quality after the rhFSH treatment [23]. These findings may indicate an increase in oocyte fertility and pregnancy rate after the FSH treatment.

Fertilization with sperm containing fragmented DNA may result in reduced sperm fertility, failure of In Vitro Fertilization (IVF) results, impaired fetal quality, decreased fetal growth, and increased early abortion [24]. Consistent with our findings, Forešta et al. showed that rhFSH was an effective drug for the treatment of male infertility and had only a few side effects. FSH has been reported to act in the early stages of spermatogenesis. It has an increasing impact on spermatogonia and increases the lifetime of immature germ cells [25]. In the present study, we found that sperm quality significantly increased in infertile oligozoospermia patients with a low FSH level receiving the rhFSH treatment. In 2012, Colacurci showed that three months of the rhFSH treatment significantly decreased sperm DNA fragmentation in oligoasthenoteratozoospermic patients with a low semen concentration (>15%) [11], but significantly increased the DFI level in patients with high basal DFI values (>15%). In contrast, we observed that the DFI level significantly reduced in oligozoospermic patients with DFI values >30% and a low hormonal level. Many researchers have shown that DNA deficiency reduces the rate of natural fertility and assisted reproduction, and that DNA fragmentation may increase pregnancy failure [26, 27].

Santi et al. concluded that FSH treatment in azoospermia men could increase pregnancy rate with ART [23].

However, the data on the relationship between DNA fragmentation and fertilization is still inconsistent and needs further study [28, 29]. In previous studies, different doses of rhFSH were used in oligozoospermic patients [30, 31]. Foresta studies showed that the rhFSH treatment at a dose of 50 IU did not increase sperm concentration. In contrast, the rhFSH treatment at a dose of 100 IU significantly increased sperm concentration in our study [11]. The spermatogenesis period of 74 days has been reported for humans. Therefore, the effective duration for rhFSH treatment would be three months [32, 33]. In this study, the rhFSH treatment at a dose of 75 IU was implemented for three months, which showed significant changes in the sperm parameters (sperm concentration, motility, and morphology), as well as in the DFI, ROS, and MDA levels.

Ethical Considerations

Compliance with ethical guidelines

The trial design was approved by ACECR biomedical research Ethics Committee (IR.ACECR.JDM.REC1397.014) and was registered in the Iranian Registry of Clinical Trial website (www.IRCT.IR) for clinical trials registration (<http://www.IRCT.IR:IRCT20170923036334N2>). All patients were informed about the study and they provided written consent form.

Animal studies: This article does not contain any study with animal participants that was performed by any of the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors contribution's

Conceptualization: Atefeh Verdi; Formal analysis: Rahi Jannatifar; Investigation: Atefeh Verdi, Seyedeh Saeideh Sahraei, Elham Asa, Rahil Janatifar; Methodology: Atefeh Verdi, Mohammad Bagher Masaeimanesh; Supervision: Atefeh Verdi; Writing—original draft preparation: Atefeh Verdi, Seyedeh Saeideh Sahraei; Writing - review & editing: Atefeh Verdi, Seyedeh Saeideh Sahraei; All authors have read and approved the final version of the manuscript. The corresponding author had full access to all of the data

in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The authors would like to thank the ACECR center for infertility treatment (Qom Branch) for supporting this research project.

Reference

- [1] Haghghian HK, Haidari F, Mohammadi-Asl J, Dadfar M. Randomized, triple-blind, placebo-controlled clinical trial examining the effects of alpha-lipoic acid supplement on the spermatogram and seminal oxidative stress in infertile men. *Fertil Steril*. 2015; 104(2):318-24. [DOI:10.1016/j.fertnstert.2015.05.014] [PMID]
- [2] Agarwal A, Tadros H, Panicker A, Tvrdá E. Role of oxidants and antioxidants in male reproduction. In: Armstrong D, Stratton RD (editors). *Oxidative Stress and Antioxidant Protection: The Science of Free Radical Biology and Disease*. Hoboken NJ: Wiley; 2016. [DOI:10.1002/9781118832431.ch15]
- [3] Takeshima T, Kuroda S, Yumura Y. Reactive oxygen species and sperm cells. In: Cristiana F, Elena A (editors). *Reactive Oxygen Species (ROS) in Living Cells*. London: Intechopen; 2018. [DOI:10.5772/intechopen.73037]
- [4] Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal Biochem*. 2017; 524:13-30. [DOI:10.1016/j.ab.2016.10.021] [PMID]
- [5] Gomez E, Irvine DS, Aitken RJ. Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. *Int J Androl*. 1998; 21(2):81-94. [DOI:10.1046/j.1365-2605.1998.00106.x] [PMID]
- [6] Fraczek M, Szkutnik D, Sanocka D, Kurpisz M. Peroxidation components of sperm lipid membranes in male infertility. *Ginekologia Polska*. 2001; 72(2):73-9.
- [7] Tavalae M, Razavi S, Nasr-Esfahani MH. Influence of sperm chromatin anomalies on assisted reproductive technology outcome. *Fertil Steril*. 2009; 91(4):1119-26. [DOI:10.1016/j.fertnstert.2008.01.063] [PMID]
- [8] Petersen CG, Mauri AL, Vagnini LD, Renzi A, Petersen B, Mattila M, et al. The effects of male age on sperm DNA damage: An evaluation of 2,178 semen samples. *JBRA Assist Reprod*. 2018; 22(4):323.
- [9] Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: A new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. *Eur Urol*. 2016; 70(4):635-45. [DOI:10.1016/j.eururo.2016.04.010] [PMID]
- [10] Barbonetti A, Calogero A, Balercia G, Garolla A, Krausz C, La Vignera S, et al. The use of follicle stimulating hormone (FSH)

- for the treatment of the infertile man: Position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). *J Endocrinol Invest.* 2018; 41(9):1107-22. [DOI:10.1007/s40618-018-0843-y] [PMID]
- [11] Colacurci N, Monti MG, Fornaro F, Izzo G, Izzo P, Trotta C, et al. Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. *J Androl.* 2012; 33(4):588-93. [DOI:10.2164/jandrol.111.013326] [PMID]
- [12] Garolla A, Ghezzi M, Cosci I, Sartini B, Bottacin A, Engl B, et al. FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. *Endocrine.* 2017; 56(2):416-25. [DOI:10.1007/s12020-016-1037-z] [PMID]
- [13] Simoni M, Santi D. FSH Treatment of male idiopathic infertility: Time for a paradigm change. *Andrology.* 2019; 8(3):535-44. [DOI:10.1111/andr.12746] [PMID]
- [14] Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update.* 2010; 16(3):231-45. [DOI:10.1093/humupd/dmp048] [PMID]
- [15] Sun TC, Zhang Y, Li HT, Liu XM, Yi DX, Tian L, et al. Sperm DNA fragmentation index, as measured by sperm chromatin dispersion, might not predict assisted reproductive outcome. *Taiwan J Obstet Gynecol.* 2018; 57(4):493-8. [DOI:10.1016/j.tjog.2018.06.003] [PMID]
- [16] Mahfouz RZ, du Plessis SS, Aziz N, Sharma R, Sabanegh E, Agarwal A. Sperm viability, apoptosis, and intracellular reactive oxygen species levels in human spermatozoa before and after induction of oxidative stress. *Fertil Steril.* 2010; 93(3):814-21. [DOI:10.1016/j.fertnstert.2008.10.068] [PMID]
- [17] Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. *Nat Revs Urol.* 2017; 14(8):470. [DOI:10.1038/nrurol.2017.69] [PMID]
- [18] Hanafy S, Halawa FA, Mostafa T, Mikhael NW, Khalil KT. Serum leptin correlates in infertile oligozoospermic males. *Andrologia.* 2007; 39:177-80. [DOI:10.1111/j.1439-0272.2007.00779.x] [PMID]
- [19] Bibov MY, Kuzmin AV, Alexandrova AA, Chistyakov VA, Dobaeva NM, Kundupyan OL. Role of the reactive oxygen species induced DNA damage in human spermatozoa dysfunction. *AME Med J.* 2018; 3(19):1-12. [DOI:10.21037/amj.2018.01.06]
- [20] Sanocka D, Kurpisz M. Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol.* 2004; 2:12. [DOI:10.1186/1477-7827-2-12] [PMID] [PMCID]
- [21] 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. *Front Endocrinol.* 2018; 9:383. [DOI:10.3389/fendo.2018.00383] [PMID] [PMCID]
- [22] Wang Y-X, Wang P, Feng W, Liu C, Yang P, Chen Y-J, et al. Relationships between seminal plasma metals/metalloids and semen quality, sperm apoptosis and DNA integrity. *Environ Pollut.* 2017; 224:224-34. [DOI:10.1016/j.envpol.2017.01.083] [PMID]
- [23] Santi D, Granata A, Simoni M. FSH treatment of male idiopathic infertility improves pregnancy rate: A meta-analysis. *Endocr Connect.* 2015; 4(3):R46-R58. [DOI:10.1530/EC-15-0050] [PMID] [PMCID]
- [24] Ollero M, Gil-Guzman E, Lopez MC, Sharma RK, Agarwal A, Larson K, et al. Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility. *Hum Reprod.* 2001; 16(9):1912-21. [DOI:10.1093/humrep/16.9.1912] [PMID]
- [25] Amirzargar MA, Yavangi M, Basiri A, Hosseini Moghaddam SM, Babolhavaeji H, Amirzargar N, et al. Comparison of Recombinant Human Follicle Stimulating Hormone (rhFSH), Human Chorionic Gonadotropin (HCG) and Human Menopausal Gonadotropin (HMG) on semen parameters after varicocele: A randomized clinical trial. *Iran J Reprod Med.* 2012; 10(5):441-52.
- [26] Foresta C, Bettella A, Garolla A, Ambrosini G, Ferlin A. Treatment of male idiopathic infertility with recombinant human follicle-stimulating hormone: A prospective, controlled, randomized clinical study. *Fertil Steril.* 2005; 84(3):654-61. [DOI:10.1016/j.fertnstert.2005.03.055] [PMID]
- [27] Ruvolo G, Roccheri MC, Brucculeri AM, Longobardi S, Cittadini E, Bosco L. Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotropic hypogonadism. *J Assist Reprod Genet.* 2013; 30(4):497-503. [DOI:10.1007/s10815-013-9951-y] [PMID] [PMCID]
- [28] Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: A systematic review and meta-analysis. *Hum Reprod.* 2012; 27(10):2908-17. [DOI:10.1093/humrep/des261] [PMID]
- [29] Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, Kammoun M, Meni-aoui I, Sallem A, et al. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. *Fertil Steril.* 2016; 105(1):58-64. [DOI:10.1016/j.fertnstert.2015.09.041] [PMID]
- [30] Zini A. Are sperm chromatin and DNA defects relevant in the clinic? *Syst Biol in Reprod Med.* 2011; 57(1-2):78-85. [DOI:10.3109/19396368.2010.515704] [PMID]
- [31] Practice Committee of the American Society for Reproductive M. The clinical utility of sperm DNA integrity testing: a guideline. *Fertil Steril.* 2013; 99(3):673-7. [DOI:10.1016/j.fertnstert.2012.12.049] [PMID]
- [32] Valenti D, La Vignera S, Condorelli RA, Rago R, Barone N, Vicari E, et al. Follicle-stimulating hormone treatment in normogonadotropic infertile men. *Nat Rev Urol.* 2013; 10(1):55. [DOI:10.1038/nrurol.2012.234] [PMID]
- [33] Aljuhayman A, Almardawi A, Fallatah M, Alhathal N. Efficacy of follicle-stimulating hormone as a treatment of severe idiopathic oligospermia: A retrospective study. *Urol Ann.* 2020; 12(1):69. [DOI:10.4103/UA.UA_37_19] [PMID] [PMCID]
- [34] Paradisi R, Busacchi P, Seracchioli R, Porcu E, Venturoli S. Effects of high doses of recombinant human follicle-stimulating hormone in the treatment of male factor infertility: Results of a pilot study. *Fertil Steril.* 2006; 86(3):728-31. [DOI:10.1016/j.fertnstert.2006.02.087] [PMID]
- [35] Schisterman EF, Sjaarda LA, Clemons T, Carrell DT, Perkins NJ, Johnstone E, et al. Effect of Folic Acid and Zinc Supplementation in Men on Semen Quality and Live Birth Among Couples Undergoing Infertility Treatment: A Randomized Clinical Trial. *Jama.* 2020; 323(1):35-48. [DOI:10.1001/jama.2019.18714]

This Page Intentionally Left Blank
