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Research Article

Evaluation of the Combination of Recombinant FSH and Antioxidant Therapy on DNA Fragmentation Index (DFI) in Infertile Men

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Introduction. Medications such as antioxidants and recombinant FSH are prescribed to improve sperm DFI (DNA fragmentation index) and treat infertility. Due to the high cost and duration of medications, in this study, we decided to investigate the effectiveness of combining these two medications with lower doses and in less time, compared to previous studies prescribing them individually, to reduce sperm DFI in infertile men. *Methods*. This study, a retrospective observational before and after trial, included a group ($n = 94$) of men aged 18-50 years with DFI > 20% suffering from male factor infertility in an andrology clinic in Tehran, Iran, from 2019 to 2020. Patients received the combination of two medications, recombinant FSH every 5 days (75 IU subcutaneously) and an antioxidant every day (selenium, vitamin E, CoQ10, and folic acid) for 2 months. The patient's sperm DFI and sperm parameters have been measured before and after the medication and were reported in patient's files. In all patients, we compare the sperm DFI and sperm parameters before and after the treatment to evaluate the effect of the combination therapy. *Results*. The sperm DFI was significantly reduced (p value ≤0.001) in the patients treated by the combined medication. The sperm DFI improvement was 50.16% in the total population. However, there were no significant changes in other sperm parameters such as sperm concentration, total motility, progressive motility, immotile sperms, and abnormal morphology. Total sperm count and nonprogressive motility had significant changes statistically. *Conclusion*. Sperm DFI is an important indicator for evaluating the quality of semen. In this study, we demonstrate that the combination of recombinant FSH and antioxidants in a shorter time and with lower doses is effective in reducing DFI significantly. But in terms of other sperm parameters, it does not make a significant difference.

1. Introduction

Infertility in men is one of the most common diseases in the world, affecting 10 to 15% of adult men [1]. Also, 50% of infertile couples suffer from infertility in men. However, the cause of infertility is often unknown [2]. Infertility in men can be the result of various reasons such as reduction in sperm count, DNA damage in sperm, lifestyle, obesity, and age [3] [4]. In most cases, for the treatment of infertility in men, assisted reproductive technologies (ART) are used, in which patients' spouses are treated with hormone therapy even though they do not have fertility problems [5].

One of the main reasons for infertility in men is the destruction of sperm DNA, which can be caused by oxidative stress. There is no DNA correction system in human sperm, and this damaged DNA may be passed on to the next generation. Moreover, DNA damage is clearly associated with infertility and failure in natural and artificial fertility [6]. Therefore, the DNA fragmentation index (DFI) was considered as one of the main tests in sperm evaluation. According to research, if the DFI is more than 20%, the probability of fertility is reduced. Spermogram includes semen parameters which are also good markers to evaluate sperm quality. Indeed, a combination of spermogram tests with DFI provides useful information, which cannot be accurately obtained by spermogram alone and can help to optimize treatment strategies [7].

Medications such as antioxidants and recombinant FSH are prescribed to improve sperm DFI and treat infertility. One of the reasons that cause damage to sperm DNA is the uncontrolled production of free radicals (ROS) and oxidative stress. Therefore, studies have used antioxidants to reduce DFI, which show that antioxidants are effective in reducing DFI [8]. Antioxidants can improve sperm parameter and increase rate of pregnancy [9]. However, some studies observed no effects on sperm parameters [7]. In previous studies, antioxidant types and doses are different, but the duration of the treatment is at least 3 months in all of them [6]. On the other hand, some researchers believe that prescribing recombinant FSH increases the likelihood of fertility with different mechanisms. FSH has a synergistic effect with testosterone in promoting germ cell survival, leading to the possibility of improved sperm DNA integrity [10]. Previous research showed that FSH administration in men with idiopathic infertility increased DNA integrity significantly [11, 12]. It is possible that recombinant FSH reduced sperm DFI by stabilizing the microenvironments and making the sperm pathways safer in men with idiopathic infertility. Moreover, recombinant FSH reported to reduce sperm DFI in functional hypogonadotropic hypogonadism by improving spermatogenesis [13]. Recombinant FSH is not effective on everyone because of different genotypes [12]. In previous studies, recombinant FSH prescribed 150 IU every other day for 3 months to reduce the sperm DFI [14].

Combination therapy in comparison with individual therapy has some advantages such as lower dosage of drugs and shorter treatment period. However, there are no studies in the literature to evaluate the effect of the combination of recombinant FSH and antioxidants on sperm parameters and DFI. Considering that many reproductive-aged couples suffer from infertility in men and due to the high cost and long duration of treatment, in this observational study, we aim to evaluate the effects of combining lower doses of recombinant FSH and antioxidants in 2 months on sperm DFI improvement in infertile men.

2. Material and Methods

2.1. Study Design. This is a retrospective, observational, and before-and-after study and was conducted in the andrology clinic of Avicenna Infertility and Recurrent Abortion Treatment Center, Tehran, Iran. The study protocol is approved by the Research Ethics Committee of Avicenna Research Institute (IR.ACECR.AVICENNA.REC.1399.033) and the Research Ethics Committee of Tehran University of Medical Sciences (approval ID: IR.TUMS.TIPS.REC.1400.007).

Due to the limitations of studies in this field, the sample size is calculated based on data obtained from pilot measurements as follows: using Glower software with a type I error of 0.05 and type 2 error of 0.2 for a mean DFI of 0.35 before and 0.45 after the study with a standard deviation of 0.25 to process before and after data bilaterally. As a result, a sample size of at least 65 patients was considered for this study.

2.2. Patients. This study stems from the extensive database that has been created using records to analyze the improvement of the patients who attended to the andrology clinic in Tehran, Iran, for infertility problems from 2019 to 2020.

We considered these criteria to include patients in the study: infertile men with the age of 18 years and above that their recorded DFI, before the treatment, were more than 20% and using the specific treatment (which is explained in the "treatment" part) for 2 months.

We excluded the male infertile patients who had the history of azoospermia, varicocele, cryptorchidism, or endocrine hypogonadism (defined by abnormal hormone levels); karyotype anomalies (including Y chromosome microdeletion); and a history of radiation and/or chemotherapy. Considering all the criteria, in conclusion, we reach to 94 out of 922 patients to enroll in our retrospective, observational study.

2.3. Treatment. All enrolled patients received the specific treatment consisting of recombinant FSH, subcutaneously administered at 75 IU dosage every five days, plus daily antioxidant supplements, for a total period of 2 months.

The antioxidant supplementation contained the following active ingredients: 60 μg selenium, 200 IU vitamin E, 5 mg folic acid, and 100 mg Q10.

In records, semen parameters and sperm DFI were evaluated in a standard semen analysis and standard sperm DFI analysis at the beginning of the treatment and after completing 2 months of therapy. Variables taken into consideration were sperm DFI, sperm chromatin immaturity, sperm concentration, total sperm count, progressive motility, nonprogressive motility, total motility, immotile, and abnormal morphology.

2.4. Other Measurements. Demographic information including age, medical, occupational, family history, lifetime history of smoke, alcohol, and opioid were recorded in patient's files. Endocrine function tests such as serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and testosterone were assessed using ELISA biochemical kits in clinic's laboratories and were reported in the patient's files.

Body weight was measured to the nearest 0.5 kg using digital scales with participants minimally clothed and barefoot. Height was measured while participants were in a standing position without shoes, using a nonstretch tape

meter fixed to a wall, and was recorded to the nearest 0.5 cm. Body mass index (BMI) was calculated as kilogram per meter squared. Weight, height, and BMI were recorded in patient's files.

2.5. Sperm DFI Detection. This test was performed using an SDFA kit (Dain Bioassay, Iran) according to the manufacturer's instructions. Briefly, a 50 μL semen was diluted in Ham's F10 medium, and semen aliquot was mixed with 50 μL agarose (6.5%). Then, 20 μL of the mixture was loaded onto a pretreated glass slide and placed on a cold surface at 4°C for 5 min. Then, the slides were treated with denaturizing solution for 7 min and then treated with lysing solution for 15 min. Following this step, the slides were washed with distilled water for 5 min, dehydration was performed using increasing concentrations of ethanol (70%, 90%, and 100%), and finally, the air-dried slide was stained. At least 200 sperms were assessed on a microscope with 100x magnification. Sperms with a large or medium halo were classified as intact chromatin, and those with no halo or a small halo were classified as sperm with fragmented DNA. The results were presented as sperm DNA fragmentation index (DFI) [15].

2.6. Semen Routine Analysis. Semen specimens were obtained from 94 infertile men who were referred to the Avicenna Infertility Clinic affiliated with the Avicenna Research Institute (ARI), Tehran, Iran.

The study was approved by the bioethics committees of the ARI. Informed consent was obtained from each healthy donor. Semen samples were collected after 48-72 h of sexual abstinence, and semen analysis was performed according to the World Health Organization guideline manual to determine semen volume, pH, motility, morphology, and sperm concentration. For the analysis of sperm motility, the computer-assisted semen analysis system was used [15].

2.7. Statistical Analysis. The baseline characteristics of participants in a study group are summarized using mean ± (standard deviation (SD)) or median ± (interquartile range (IQR)) for continuous variables and frequency (%) for categorical variables. The normality of data was verified using the Kolmogorov-Smirnov test. The result of Kolmogorov-Smirnov test shows that only sperm concentration follows the normal distribution. Differences between each parameter (DFI and semen parameters), before and after treatment, have been verified using the two-tailed paired t-test (for variables with normal distribution) and the Wilcoxon test (for variables without normal distribution). The null hypothesis to determine the efficacy is that there would be no change in DFI after the treatment. The statistical significance level is defined as a two-tailed p value of 0.05. All statistical analyses were conducted using SPSS Version 22.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Patient Characteristics. The 94 men are enrolled in accordance with the inclusion and exclusion criteria. The average age of the participants is 38.9 (5.8) years old (range 27-50 years). Baseline characteristics of men in the treatment group such as BMI, smoking behavior, alcohol, and substance use are shown in Table 1.

Table 1: Baseline characteristics of participants in a study group at enrollment.

Variables	Participants ($n = 94$)
Age (years) (mean (SD))	38.9 (5.8)
BMI (kg/m^2) (mean (SD))	27.4 (3.9)
Height (cm) (mean (SD))	176.9 (5.6)
Weight (kg) (mean ± (SD))	85.6 (13.1)
Smoker (N) (%)	7 (7.5%)
Substance abuser (N) (%)	2 (2.1%)
Alcohol consumption (N) (%)	1 (1%)

BMI: body mass index; SD: standard deviation; N: number.

3.2. Effect of Combination Therapy on DFI. The DFI median for all patients at baseline is 32.5 (17.0). The result suggests that after two months of the treatment, the DFI decreased to 15.0 (10.2). This DFI reduction is statistically significant with p value less than 0.001 (Table 2). The mean (SD) of DFI reduction is 50.16 (0.21). Seven out of 94 patients have visited the clinic after 3 months of treatment as well. On average, the DFI of these patients significantly reduced after 2 months (p value = 0.004) and also after 3 months (p value = 0.002). However, the reduction from month 2 to month 3 is not significant (p value = 0.29). Please note that patients instructed to stop the treatment after their second visit if their DFI dropped below 20%. To investigate the effect of age on the DFI reduction, we classified the participants into three groups: 20-29 years, 30-39 years, and over 40 years. The result of this analysis is presented in Figure 1. As seen in the figures, in all age categories, we observe a significant decrease in DFI. The biggest decrease is seen in the age group 20-29. We also investigate the effect of BMI on the DFI reduction. The result is reported in Figure S1. Our analysis found no significant differences in sperm chromatin immaturity ($p = 0.130$) (Figure 2).

3.3. Effect of Combination Therapy on Seminal Parameters. We also analyze the effect of the combined antioxidant and recombinant FSH on different semen parameters. The results of this analysis are demonstrated in Table 2. As seen in the table, there is no significant change in sperm chromatin immaturity ($p = 0.138$) and sperm concentration ($p = 0.3$) after the treatment. However, we observe a significant reduction in total sperm count after the treatment ($p \leq 0.001$). Also, the result shows no significant changes in the total motility ($p = 0.12$), progressive motility ($p = 0.658$), immotile sperms ($p = 0.1$), and abnormal morphology ($p = 0.58$). However, we observe a significant increase in nonprogressive motility ($p = 0.003$).

We divide the patients into two groups of normal and abnormal using World Health Organization (WHO) guideline and reference values [16]. The reference limit for total sperm count is 39×10^6. A patient is categorized as normal if their total sperm count is equal or above the reference

Table 2: Descriptive statistics of semen and sperm DFI analysis before and after the treatment.

Variables	Total participants ($n = 94$)		
	Before	After	p value
Sperm DNA fragmentation index (DFI) (%) (median (IQR))	32.5 (17.0)	15.0 (10.2)	≤0.001*[b]
Sperm chromatin immaturity (%) (median (IQR))	11.0 (7.0)	9.0 (9.0)	0.138[b]
Sperm concentration (10^6/ml) (mean (SD))	26.9 (15.0)	25.9 (13.9)	0.300[a]
Total sperm count (10^6) (median (IQR))	70.0 (68.0)	44.9 (41.3)	0.006*[b]
Total motility (%) (median (IQR))	50.0 (20.0)	50.0 (15.0)	0.120[b]
Progressive motility (%) (median (IQR))	25.0 (15.0)	22.5 (15.0)	0.658[b]
Nonprogressive motility (%) (median (IQR))	25.0 (10.0)	25.0 (5.0)	0.002*[b]
Immotile sperms (%) (median (IQR))	50.0 (20.0)	50.0 (15.0)	0.100[b]
Abnormal morphology (%) (median (IQR))	99.00 (2.00)	99.00 (1.00)	0.580[b]

[a]Normal distribution, paired t-test, [b]not normal distribution, Wilcoxon test, *considered as statistically significant.

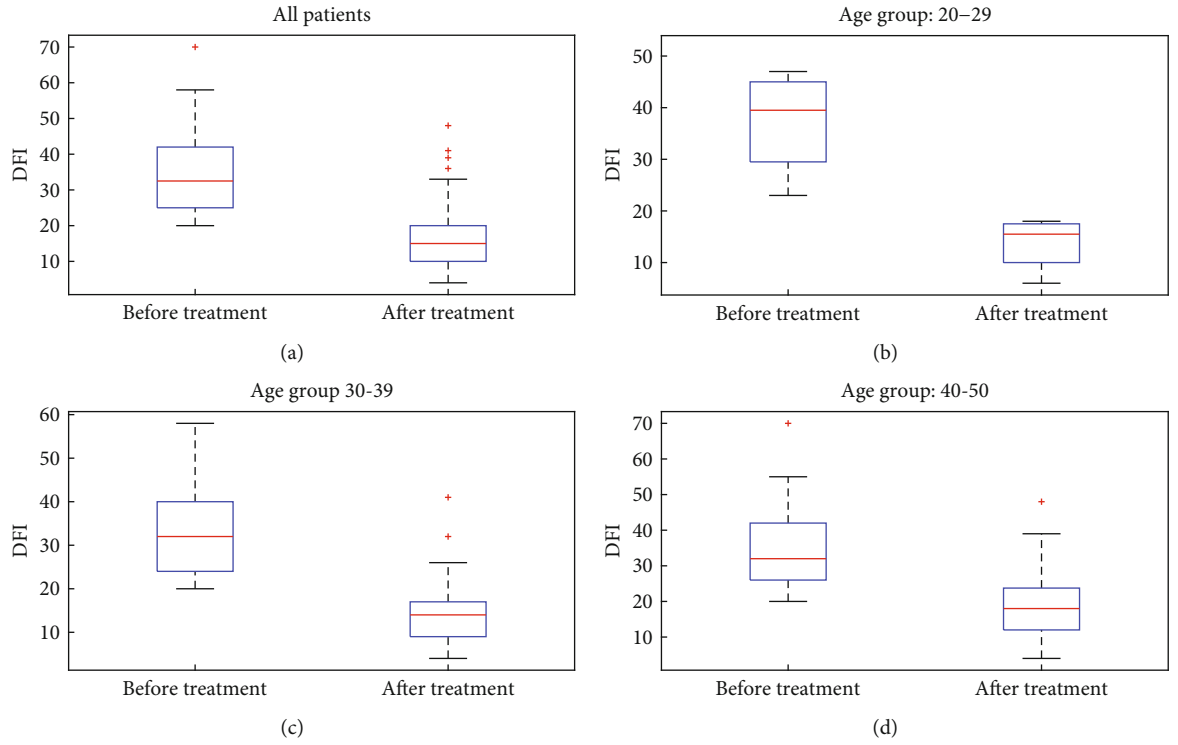

Figure 1: Effect of age on DFI change. The box plots represent the distribution of DFI before and after the treatment for (a) all patients (94 patients, p value = 1.02e − 26) (b) patients within the age group of 20-29 (6 patients, p value = 1.06e − 3), (c) patients within the age group of 30-39 (47 patients, p value = 1.11e − 16), and (d) patients within the age group of 40-50 (41 patients, p value = 8.29e − 10).

value. Otherwise, a patient is categorized as abnormal. We investigate if the treatment affects the patient group. For this purpose, we evaluate if the patient group is changed from normal to abnormal or vice versa after the treatment. The result of this analysis is presented in Table 3. As seen in the table, 16 patients that were in the normal range moves to abnormal range, and 5 patients from abnormal range transferred to the normal range after the treatment.

As reported in Table 2, we observed a significant increase in nonprogressive motility ($p = 0.002$). We investigated if the significant increase in nonprogressive motility is due to the change of status of the immotile or progressive sperms. For this purpose, we use heat map to visualize the change of progressive, nonprogressive, and immotile data of each patient before and after the treatment. Figure 3 shows the result of this analysis. As seen in the figure, non-progressive motility is increased in 39 patients. In 29 patients (with the age mean of 38.7), and the immotile sperms have been decreased which means that they have been transformed to nonprogressive sperms. On the other hand, in 10 patients (with the age mean of 42.2), the progressive sperms have been transformed to nonprogressive sperms. Based on this result, the average age of patients who have sperm motility development is less than those having sperm motility regression.

Based on Table 2, there are no significant differences in progressive motility before and after the intervention

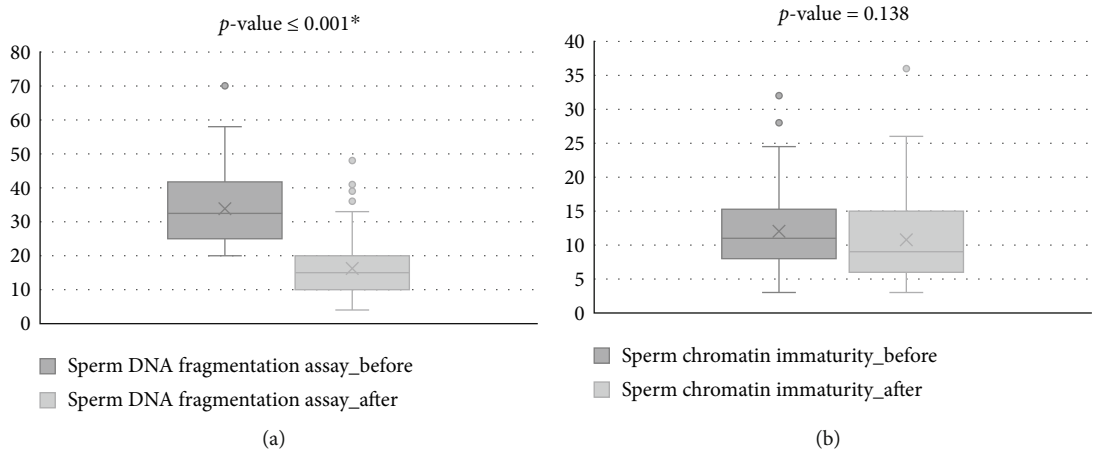

Figure 2: Effect of treatment on sperm DFI and sperm chromatin immaturity. (a) shows the range of sperm DFI, and (b) presents the sperm chromatin immaturity before (dark grey) and after (light grey) of treatment.

Table 3: Number of patients with total sperm count changes before and after the intervention.

McNemar's test p value = 0.029		Total sperm count—after	
		Normal[a]	Abnormal[b]
Total sperm count—before	Normal[a]	50	16
	Abnormal[b]	5	23

[a]Total sperm count $\geq 39 \times 10^6$; [b]total sperm count $< 39 \times 10^6$ (cut-off point $= 39 \times 10^6$ based on WHO guideline).

Figure 3: Heat map to present motile and immotile sperm changes. Each column represents a patient, and rows represent the change of progressive, nonprogressive, and immotile sperms. The colors show the changes of different types of motilities after the intervention. The yellow color shows the increase in sperm motility after the treatment, and the green color shows the reduction of sperm motility after the treatment.

($p = 0.658$). Also, the overall results reveal no significant differences in total motility ($p = 0.120$), immotile sperms ($p = 0.100$), and abnormal morphology ($p = 0.633$).

4. Discussion

To the best of our knowledge, this study is the first study to examine the effect of the combination of recombinant FSH and antioxidants on DFI and sperm parameters in infertile men. In this study, we demonstrate that this combination therapy, with lower doses for 2 months, is effective to induce a significant reduction in sperm DFI, but there is no significant improvement on other semen parameters.

The DNA fragmentation index (DFI) is a well-known marker of sperm quality, and the DFI higher than 20% can increase the risk of infertility in men. Medications, which reduce DFI to less than 20%, increase fertility and pregnancy rate. Semen parameters are also good markers to evaluate sperm quality [17].

A recombinant FSH plays a pivotal role in sperm DNA maturation and stabilizing sperm environment. Also, the FSH is a key regulator of testis function, required for the establishment of full postnatal development of Sertoli and germ cells and for the maintenance of spermatogenesis in adult men [10]. The suggested dose in studies is 150 IU once daily or every other day for 3 months [14] [12]. For example, Colacurci et al. showed that by prescribing Gonal-f® 150 IU every other day, within 3 months, the DFI decreased by 35.4%, and other sperm parameters improved [13]. In the present study, we suggest recombinant FSH with a lower dose, 75 IU every five days, in combination with antioxidants for 2 months.

Several studies have reported that high levels of ROS recognized as an effective factor in male infertility are associated with sperm DNA damage [18]. At low levels, ROS plays a significant role in many biological processes such as capacitation, hyperactivation, and acrosome reaction, which is essential for successful fertilization [19]. ROS are known to have a negative impact on sperm quality by causing DNA damage and reducing sperm motility. Antioxidant treatment may have helped to mitigate the negative effects of ROS on sperm quality by reducing the overall level of ROS in the semen. However, the antioxidant treatment alone may not have been sufficient to fully reverse the damage already done to the sperm DNA, leading to a reduced percentage of abnormal spermatozoa. The suggested medicines and their doses are different in each study, but the duration of the treatments is at least 3 months [9] [8]. For example, Salehi showed that by prescribing antioxidant supplementation containing 50 mg vitamin E, 500 mg vitamin C, and 100 mg CoQ10 daily for 3 months, the DFI improvement rate was 38.9%, and other sperm parameters improved too [6]. In the present study, we recommended 60 μg selenium, 200 IU vitamin E, 100 mg CoQ10, and 5 mg folic acid per day to have in combination with recombinant FSH for 2 months.

Table 4: Therapeutic schemes of different methods and their effect on sperm parameters.

Treatment method	Medication	Duration (month)	DFI decrease (%)	Total sperm count	Sperm motility	Normal morphology	Reference
Hormone therapy	r-FSH (150 IU, every other day)	3	35.4% [1] 12% [2]	↑* [1] ↑* [2]	Total ↑* [1] Progressive↑* [2]	↑ [1] ↑ [2]	[1] [13] [2] [14]
	r-FSH (150 IU, daily)	3	24.58%	↓	All ↓	—	[12]
Antioxidant therapy	50 mg vitamin E, 500 mg vitamin C, 100 mg Q10	3	38.9%	—	Total ↑* Progressive ↑*	↓*	[6]
	N/A	3	28.6%	—	—	—	[8]
	30 mg vitamin C, 5 mg vitamin E, 25 μg selenium, 5 mg zinc	3	0%	↓	Total ↑ Progressive ↓	↑	[7]
Combination therapy	r-FSH (75 IU, every 5 days) + 60 μg selenium + 200 IU vitamin E + 100 mg Q10 + 5 mg folic acid	2	53.84%	↓*	Nonprog ↑* Others ↓	↑	This manuscript

↑: increase; ↓: decrease; *significant change.

On the other hand, FSH is a key regulator of spermatogenesis, which is the process by which sperm cells are formed. It is known to have a positive effect on sperm quality by promoting the production of healthy sperm cells. This may have contributed to the improvement in DFI. Our study provides evident that the combination of FSH and antioxidant treatment may have had a synergistic effect on sperm quality, with the FSH promoting the production of healthy sperm cells and the antioxidants reducing the negative effects of ROS.

For this study, since we do not have any control group, we compare the results with previous studies presented in Table 4. As shown in Table 4, the response to FSH therapy and antioxidant therapy can be variable and may depend on a variety of factors such as study design, population, or treatment protocol (dose and duration of treatment). Additionally, other factors such as genetic and environmental factors may have played a role in these parameters. In this study, the rate of DFI reduction is 50.16%, which indicates that sperm quality can improve with the combination treatment of recombinant FSH and antioxidant, with a lower dose and shorter time. In semen parameters, total sperm count and nonprogressive motility have a significant difference before and after the treatment. Other sperm parameters such as sperm concentration, total motility, progressive motility, immotile sperms, and abnormal morphology do not change significantly with this intervention. These results may suggest that the proposed therapy impacts the posttesticular factors which mostly influence DFI. While the significant improvement in DFI is observed, the reduction in oxidative stress and ROS levels was not enough to produce a significant improvement in the proportion of abnormal sperm. It is possible that the dose or duration of FSH treatment used in our study may have been insufficient to elicit a significant improvement in these sperm parameters. Therefore, higher doses or longer durations of treatment may be needed to produce these effects. In any case, more research is needed to fully understand the complex relationship between ROS, antioxidants, FSH, and sperm quality and to determine the optimal treatment protocol (dosage and duration) for improving sperm parameters in infertile men.

This study has many advantages; first, using the combination of two different medications with two different mechanisms can improve DFI as the primary focus of this study, with no improvement on other semen parameters. The sperm quality is better than individual therapy. Second, using lower doses of each medication in the combination therapy is compared to individual therapy. Third, duration of the treatment decreases to 2 months in combination therapy. Fourth, the sample size of cases is large which provided the opportunity to produce reliable results.

The primary focus of this retrospective study was to evaluate the effect of antioxidant and recombinant FSH treatment on DFI and the sperm parameters such as sperm concentration, as secondary outcome. However, this study has some limitations which must be acknowledged: first, the lack of a control group; second, the lack of evaluating pregnancy rate following the treatment; third, missing antioxidant level and concentration of sex hormones after the treatment. These types of data collections would require a prospective study design in which patients are followed over time to track pregnancy outcomes and other factors that provide valuable information about the effectiveness of treatment.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Figure S1: effect of BMI on DFI change. The box plots represent the distribution of DFI before and after the treatment for (a) all patients (94 patients, $p\,value = 3.37e - 26$) and (b) patients with BMI. *(Supplementary Materials)*

References

[1] V. A. O'brien, "The genetic causes of male factor infertility: a review," *Fertility and Sterility*, vol. 93, no. 1, pp. 1–12, 2010.

[2] S. A. Kumar and A. K. Singh, "Trends of male factor infertility, an important cause of infertility: a review of literature," *Journal of Human Reproductive Sciences*, vol. 8, no. 4, pp. 191–196, 2015.

[3] H. A. Belloc, A. Hazout, A. Zini et al., "How to overcome male infertility after 40: influence of paternal age on fertility," *Maturitas*, vol. 78, no. 1, pp. 22–29, 2014.

[4] J. O. Rufus, "Male obesity and semen quality: any association?," *International Journal of Reproductive Biomedicine*, vol. 16, no. 4, p. 285, 2018.

[5] S. T. Nardelli, "Assisted reproductive technologies (ARTs): evaluation of evidence to support public policy development," *Reproductive Health*, vol. 11, no. 1, pp. 1–4, 2014.

[6] S. S. Salehi, "Effect of antioxidant therapy on the sperm DNA integrity improvement; a longitudinal cohort study," *International Journal of Reproductive Biomedicine*, vol. 17, no. 2, p. 99, 2019.

[7] O. K. Stenqvist, K. Oleszczuk, I. Leijonhufvud, and A. Giwercman, "Impact of antioxidant treatment on DNA fragmentation index: a double-blind placebo-controlled randomized trial," *Andrology*, vol. 6, no. 6, pp. 811–816, 2018.

[8] K. I. Le Saint, "Beneficial effect of antioxidant therapy on sperm DNA integrity is not associated with a similar effect on sperm chromatin integrity," *AME Medical Journal*, vol. 4, no. 31, 2019.

[9] D. G. Busetto, F. del Giudice, A. Virmani et al., "Body mass index and age correlate with antioxidant supplementation effects on sperm quality: post hoc analyses from a double-blind placebo-controlled trial," *Andrologia*, vol. 52, no. 3, article e13523, 2020.

[10] R. M. Ruvolo, M. C. Roccheri, A. M. Brucculeri, S. Longobardi, E. Cittadini, and L. Bosco, "Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotropic hypogonadism," *Journal of Assisted Reproduction and Genetics*, vol. 30, no. 4, pp. 497–503, 2013.

[11] Nicola Colacurci, M. G. Monti, F. Fornaro et al., "Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia," *Journal of Andrology*, vol. 33, no. 4, pp. 588–593, 2012.

[12] S. D. Simoni, D. Santi, L. Negri et al., "Treatment with human, recombinant FSH improves sperm DNA fragmentation in idiopathic infertile men depending on the FSH receptor polymorphism p. N680S: a pharmacogenetic study," *Human Reproduction*, vol. 31, no. 9, pp. 1960–1969, 2016.

[13] D. L. Colacurci, V. de Leo, G. Ruvolo et al., "Recombinant FSH improves sperm DNA damage in male infertility: a phase II clinical trial," *Frontiers in Endocrinology*, vol. 9, 2018.

[14] G. M. Garolla, M. Ghezzi, I. Cosci et al., "FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate," *Endocrine*, vol. 56, no. 2, pp. 416–425, 2017.

[15] D. S. Heidari, "Fibronectin as a new biomarker for human sperm selection in assisted reproductive technology," *Turkish Journal of Urology*, vol. 45, no. 2, p. 83, 2019.

[16] World Health Organization, *WHO Laboratory Manual for the Examination and Processing of Human Semen*, WHO Press, Geneva, Switzerland, 6th edition, 2010, Retrieved March 16, 2022, from https://www.who.int/publications/i/item/9789240030787.

[17] L.-C. K. Virro, K. L. Larson-Cook, and 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," *Fertility and Sterility*, vol. 81, no. 5, pp. 1289–1295, 2004.

[18] J. A. Lopes, A. Jurisicova, J. G. Sun, and R. F. Casper, "Reactive oxygen species: potential cause for DNA fragmentation in human spermatozoa," *Human Reproduction*, vol. 13, no. 4, pp. 896–900, 1998.

[19] R. Aitken, "Reactive oxygen species as mediators of sperm capacitation and pathological damage," *Molecular Reproduction and Development*, vol. 84, no. 10, pp. 1039–1052, 2017.