

Intracytoplasmic sperm injection outcomes after anti-oxidant treatment in repeated implantation failure

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ABSTRACT

Objectives: Aim of this study was to explore the effects of three months oral anti-oxidant treatment in normozoospermic men with high DNA fragmentation index (DFI) and recurrent intracytoplasmic sperm injection (ICSI) failures.

Methods: This is a retrospective analyses of normozoospermic patients with high DFI levels (> 30%) with at least two unsuccessful ICSI attempts. Among the participants, the study group included men who had oral anti-oxidant treatment. The men who did not receive any treatment during the study period were taken as controls. The outcomes of new ICSI cycles were compared.

Results: During the study period 24 men have used three months of oral anti-oxidant treatment before a new ICSI cycle whereas the remaining 46 had ICSI-only without any other intervention. Duration of stimulation, the number of metaphase II oocytes, fertilization rate and implantation rates were similar between two groups. Although not statistically significant, cases in anti-oxidant group have slightly better pregnancy rates compared to ICSI-only group (37.5% vs 19.6%, $p > 0.05$). Only one case in each group had abortion.

Conclusions: This study reveals that there is no statistically significant difference in the ICSI outcomes of the patients with high DFI levels, following unsuccessful ICSI attempts after oral anti-oxidant treatment. The possible benefit derived from the anti-oxidant therapy needs to be clarified with clinical studies with greater study populations.

Keywords: Oxidative stress, DNA fragmentation, intracytoplasmic sperm injection, spermatozoa

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Among reproductive age couples, approximately 15% suffer from infertility [1]. A relatively high number of women fail to have a pregnancy despite the apparent absence of a male or female factor of infertility. Normal semen analysis in these couples mandates some other analyses which address the integrity of sperm DNA. Recent studies have documented an

association between high levels of sperm DNA fragmentation and impaired fertilization/embryo development, lower implantation rates and higher miscarriages [2]. In animal studies, sperm DNA damage was found to be related with birth defects in the offsprings due to impaired DNA integrity [3]. DNA damage in spermatozoa may be induced by different



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mechanisms that include; apoptosis, abnormal chromatin packaging and post-testicular causes of sperm DNA fragmentation ensued from reactive oxygen species (ROS) during transit through the male reproductive tract, DNA fragmentation caused by endogenous endonucleases, radiotherapy and chemotherapy or environmental causes (smoking, pollution) [4].

Oxidative stress to spermatozoa is very common, affecting between 30% and 80% of infertile men [5]. Seminal plasma the primary source of ROS is spermatozoa and polymorphonuclear leukocytes. In fact production of ROS is an outgrowth of physiological process during capacitation and acrosome reaction. Yet, high levels of ROS in seminal plasma is associated with poor semen quality. In one study it has been demonstrated that ROS causes almost 4-fold higher levels of DNA fragmentation and can be treated with anti-oxidants [6].

Antioxidant treatment has been widely used and accepted treatment for men with high sperm DNA fragmentation [7]. Several studies have suggested the use of anti-oxidant therapy in the treatment of male infertility to improve semen quality, especially in DNA damage and sperm motility [8]. Aim of this study was to explore the effects of three months oral anti-oxidant treatment in men with high levels of sperm DNA fragmentation and normozoospermia and history of recurrent intracytoplasmic sperm injection (ICSI) failures.

METHODS

This is a retrospective analyses of patients who admitted to private in vitro fertilization (IVF) center between January and December 2016. This study included normozoospermic patients (according to WHO 2010 criteria) with high DFI levels (> 30%) detected by TUNEL assay. All patients had experienced at least two unsuccessful ICSI attempts with ejaculated spermatozoa. Among the participants, the study group included men who had oral anti-oxidant treatment. The men who did not receive any treatment during the study period were taken as controls. Heavy smokers (> 20 cigarettes/day) and patients with; abnormal medical history, abnormalities physical examination, impaired endocrine profile, genital infections, leukocytospermia, cancer or cancer therapies, varicocele, cryptorchidism and genetic

disorders were excluded. Couples with female partners fulfilling the Bologna criteria, preimplantation genetic screening and cryo cycles were also excluded.

All participants underwent ICSI using antagonist protocol. On day 2 of menstrual cycle, gonadotropins were started at doses ranging from 150 to 225 IU (Menopur, Ferring). On day 6 Gonadotropin-releasing hormone (GnRH) antagonist was added (0.25 mg/day, Cetrotide, Serono). The gonadotropin doses are adjusted according to patients response. When at least three follicles are > 17 mm in diameter ovulation is triggered with human chorionic gonadotropin (hCG) (Ovitrelle, Serono). Oocyte pick up is performed under sedation. The number of metaphase II (MII) oocytes, fertilization, embryo quality, and the cycle outcomes (implantation, clinical pregnancy, early pregnancy loss) of all couples were recorded. Patients in the study group underwent ICSI after receiving 3 months of anti-oxidant treatment (Proxead Plus, İstanbul, Eczacıbaşı), while ICSI was the sole treatment for controls. Pregnancy was recorded after betahCG test at day 12 after embryo transfer.

This retrospective study was conducted in accordance with the ethical principles defined in the Helsinki Declaration.

Statistical Analysis

Statistical analyses performed by IBM SPSS for Windows Version 22.0 statistical package. Continuous variables presented as mean±standard deviation or median (minimum-maximum). Categorical variables summarized as frequencies and percentages. Normality of the continuous variables was evaluated by Kolmogorov Smirnow test. Differences between the two groups according to continuous variables were determined by independent samples t-test or Mann Whitney U test as appropriate. Categorical variables were compared by Chi-square or Fisher's exact test. Univariate associations between variables were estimated by Pearson's coefficient correlation. A p value less than 0.05 was considered significant.

RESULTS

Analysis of the recorded revealed that there are 70 couples fulfilling the inclusion criteria. Among these cases 24 men have used three months of anti-oxidant

Table 1. Demographic characteristics of groups

	OA (n = 24)	ICSI-only (n = 46)	p value
Age of females (years)	33.25 ± 5.18	34.1 ± 3.68	0.423
BMI (kg/m ²)	24.33 ± 3.17	25.76 ± 2.36	0.170
Previous failed attempts (n)	3.17±1.55	3.86 ± 1.42	< 0.05
Infertility duration(years)	7.21 ± 2.78	8.2 ± 2.60	0.144
AMH (ng/ml)	2.20 ± 1.39	2.12 ± 1.16	0.810
DFI (%)	43.33 ± 5.87	41.8 ± 6.48	0.337

Data are presented as mean±standard deviation. OA = oral antioxidant, ICSI = intracytoplasmic sperm injection, BMI = Body mass index, AMH = Anti-Mullerian Hormone, DFI = DNA fragmentation index

treatment before a new ICSI cycle whereas the remaining 46 had ICSI-only. The study and control groups had comparable demographic characteristics by means of female partner's age, body mass index (BMI), duration of infertility, anti-Mullerian hormone (AMH) levels and DNA fragmentation index (DFI); suggesting that the confounding factors supposed to influence ICSI outcomes were almost matched (Table 1). The only statistically significant differences between the treatment and control groups were the number of previous failed attempts.

The cycle characteristics and outcomes are given in Table 2. Duration of stimulation, the number of MII oocytes, fertilization rate and implantation rates were similar between two groups. Although not statistically significant, cases in anti-oxidant group have slightly better pregnancy rates compared to ICSI-only group (37.5% vs 19.6%, $p > 0.05$, Table 2). Only one case in each group had abortion.

DISCUSSION

Although there have been previous studies searching for the effect of anti-oxidant treatment on sperm DNA fragmentation incidence or DFI, up to our knowledge, this is the first study that evaluates the effects of anti-oxidant treatment on ICSI outcomes in normozoospermic patients with recurrent ICSI failure who have high DFI levels.

Data in this study suggest that anti-oxidant treatment for patients with high DFI levels, following unsuccessful ICSI attempts does not offer any advantage in terms of improving the ICSI outcomes. Yet it's difficult to conclude that anti-oxidant treatment has no beneficial effect in terms of decreasing ROS formation and improving fertility. The literature on the effect of anti-oxidant treatment on DNA fragmentation is conflicting. While some studies suggested beneficial effects, others reported a lack of improvement in the

Table 2. Cycle characteristics and cycle outcomes of study and control groups

	OA (n = 24)	ICSI-only (n = 46)	p value
Stimulation duration (days)	11.26 ± 1.25	11.46 ± 1.68	0.610
No of MII oocytes	6.39 ± 4.18	7.24 ± 2.45	0.287
Fertilization rate (%)	74.30 ± 18.94	70.30 ± 20.26	0.406
Implantation rate (%)	26.50 ± 6.61	24.50 ± 8.25	0.176
Clinical pregnancy, n (%)	9 (37.5)	9 (19.6)	0.103
Abortion rate, n (%)	1 (4.2)	1 (2.2)	0.634

Data are presented as mean ± standard deviation. OA = oral antioxidant, ICSI = intracytoplasmic sperm injection, MII = metaphase II

outcomes. Menezo *et al.* [9] reported that anti-oxidant treatment led to a decrease in sperm fragmentation. An unexpected negative effect was also reported for anti-oxidant treatment which is an increase in sperm decondensation. The author concluded that this observation may be the cause of the discrepancy regarding the improvement in male fertility with anti-oxidant treatment [9]. In a similar way Amar *et al.* [10] stated that although anti-oxidant treatment limit ROS generation and related DNA damage, current anti-oxidant therapies might not be efficient and lead to decondensation and offered sequential treatment for handling the situation. On the contrary Greco *et al.* [11] suggested that antioxidant treatment reduces the proportion of spermatozoa with DNA damage and improves ICSI outcomes in men with high levels of sperm DNA fragmentation. However, similar to our study, no statistically significant difference was found in fertilization rates after anti-oxidant treatment. Of particular concern, in the study patients with no apparent improvement of ejaculated spermatozoa after the treatment were switched to an invasive alternative. Authors stated that; they did not address whether keeping up with the same treatment instead of switching to a more invasive one would make any difference and they also concluded this treatment may not be equally effective in a subgroup of patients who have no improvement after anti-oxidant treatment [11]. There are other studies consistent with the main finding of this study. Tunc *et al.* [12] reported that anti-oxidant treatment improves sperm DNA integrity. Greco *et al.* [13] reported that short term anti-oxidant supplementation is efficient for treating men with high sperm DNA damage. Abad *et al.* [14] suggested that in addition to improving semen quality in terms of key seminal parameters and basal DNA damage, anti-oxidant treatment also promotes better outcomes following assisted reproductive techniques. Gaul-Frau *et al.* [7] also showed beneficial effect of oral anti-oxidant treatment in grade 1 varicocele cases. However they also stated that despite better outcomes in terms of pregnancy by natural conception, higher rates of miscarriages were reported after oral anti-oxidant treatment [7].

Limitations

One of the limitations of our study apart from its retrospective nature is that the sample sizes were

small. Literature in general keeps up with the hypothesis that anti-oxidant oral treatment appears to decrease the amount of sperm with DNA damage. However, mostly the effects are reported after short term treatment and there is no long-term follow-up results and the endpoint that is measured is sperm DNA integrity. Besides, most of the clinical studies have limited number of participants and there is a great variation in the subpopulations. Therefore, this type of statement underlying the benefit derived from the anti-oxidant therapy needs to be balanced until more clinical studies with greater study populations are performed.

CONCLUSION

This study reveals that there is no statistically significant difference in the ICSI outcomes of the patients with high DFI levels, following unsuccessful ICSI attempts after oral anti-oxidant treatment.

Author Contribution

Research conception & design: ST, GŞÇ, AHH; Data acquisition/analysis and interpretation: EGP; MK; Statistical analysis: ST; Drafting of the manuscript: ST, GŞÇ, MK, AHH; Critical revision of the manuscript and approval of final manuscript: ST, GŞÇ, EGP, MK, AHH.

Ethics statement

Recently published The National Code on Clinical Trials has declared that ethics approval is not necessary for real retrospective studies. This is a retrospective study based on recorded data. Therefore approval from owners of the data (ST, GŞÇ, EGP and AHH) is obtained.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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