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Comparison of Intracytoplasmic Sperm Injection Outcome with Sperm Selection Techniques in Oligoasthenozoospermic Males: A Randomized Controlled Trial

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Abstract

Background: Male infertility rate has increased over the recent years across the globe. Intracytoplasmic sperm injection (ICSI) is mainly used to treat male infertility; however, in this method, sperm selection plays a critical role in improving ICSI outcome.
Objectives: The aim of this study was to evaluate the efficiency of three sperm selection techniques on ICSI outcomes in oligoasthenozoospermic men.
Methods: This randomized clinical trial study was performed on 80 oligoasthenozoospermic (OAS) couples receiving ICSI in Milad Infertility Center, Mashhad, Iran from October 2016 to June 2017. The couples were randomly assigned to the three groups of control, hypo-osmotic swelling test (HOST) and hyaluronic acid (HA) according to the sperm selection technique for ICSI. Fertilization rate, embryo development (ED), embryo quality (EQ) and implantation rate (IR) were evaluated and compared among the groups.
Results: Our results showed that there was no significant difference in fertilization rate between the studied groups. ED rate was increased in the HOST and HA groups compared to the control group, but this difference was not significant. EQ was increased in the HOST ($P = 0.001$) and HA groups ($P = 0.001$) compared to the control group. IR was enhanced in the HA group in comparison with the control group ($P = 0.021$), but there was no significant difference between the HOST and HA groups.
Conclusions: The results of this study showed that for achieving successful clinical outcomes and improved embryo quality and implantation rate in oligoasthenozoospermic males undergoing Intracytoplasmic sperm injection, effective sperm selection through either the HOST or HA technique is necessary.

Keywords: DNA Fragmentation, Fertilization, Injection, Intracytoplasmic, Male Infertility, Oligoasthenozoospermic, Selection, Sperm

1. Background

According to statistical reports, infertility occurs in 15% to 30% of couples, 20% to 30% of which are due to male factors (1). Various factors such as inadequate sperm count, semen deficiency, anatomical problems, hormonal imbalances, genetic defects, and sperm DNA fragmentation (SDF) may contribute to male infertility (2).

Today, assisted reproductive techniques (ARTs) are applied as a treatment, and in vitro fertilization (IVF) is a strategy used worldwide to help and treat infertile couples (3, 4). However, studies have shown an increase in adverse pregnancy outcomes and congenital malformations in ART infants (5).

Intracytoplasmic sperm injection (ICSI) has been the most popular approach to solve male infertility problems. However, the selection of high-quality sperms plays a critical role in ICSI outcome (6). According to previous studies, sperm fertilization with damaged DNA causes mutations in the fetus and increases the risk of cancer in the offspring. In fact, DNA damage is related to male infertility and it has a negative impact on sperm fertilizing capacity (7).

In addition, sperm with damaged DNA in IVF leads to incomplete embryonic development or the transfer of genetic defects to the offspring (8). Therefore, precise measurement of the percentage of DNA damage is impor-

tant for ARTs (9). The available methods should be facilitated for normal sperm selection to overcome IVF failures (10). Novel methods have been proposed such as using negatively-charged sperm (11) and diagnostic markers at the level of sperm membrane. Also, hyaluronic acid (HA) binding-mediated sperm selection method has been proposed for this purpose. The HA-binding ability of sperm depends on sperm receptors on the zona pellucida (12). In this regard, studies have shown a significant decrease in the SDF using HA binding for sperm selection. The application of this method might decrease genetic complications and improve general health after ICSI (13).

Another method for sperm selection based on functional assessments in infertile men is the hypo-osmotic swelling test (HOST). Disclosure of sperm to hypo-osmotic conditions leads the influx of water into the cytoplasm, thereby expanding sperm membrane. This results in the opening of the calcium channels and influx of calcium into sperm cytoplasm, leading to acrosome reaction (14). As a result of this expansion in sperm membrane, different tail swelling patterns are induced, based on which sperms are classified as grade A to grade G. In terms of healthy DNA, grade-d sperms have the highest quality and therefore, they can be used for insemination, whereas grade-g sperms have the lowest quality and therefore, their insemination must be avoided. Expanding of sperm volume indicates different grades of sperm membrane integrity and functionality and DNA integrity (15, 16). Rouen et al. also using flagella classes in HOST identified a specific type of sperm with a balanced genome (genetic content) (17).

2. Objectives

Although the selection of live healthy sperms is possible through the HOST grading method (18), the use of this method is still under discussion. With this background in mind, this study was designed to compare ICSI outcomes with those of conventional, HOST and HA methods of sperm selection in oligoasthenozoospermic men. In other words, this study was performed to determine whether the HOST method is as useful as the HA technique.

3. Methods

This parallel clinical trial was carried out to evaluate the effects of three sperm selection techniques on ICSI outcomes in oligoasthenozoospermic men. We included 80 infertile couples referred to Milad Infertility Center, Mashhad University of Medical Sciences, Mashhad, Iran from October 2016 to June 2017 due to male factor. The sample size was calculated using the appropriate statistical test and according to the project budget (19). The couples were allocated to three groups according to the table of random numbers.

The inclusion criteria for the couples were as follows: (1) infertile (oligoasthenozoospermic) men receiving ICSI for the first or second time and having a history of infertility for 2 to 10 years, (2) sperm count between 5×10^6/mL and 14×10^6/mL in natural ejaculation, (3) sperm motility rate below 30% and normal morphology more than 30%, (4) infertile men aged below 40 years, and (5) females aged between 20 and 37 years without any infertility problems and with 6 to 7 oocytes. The oocytes had the same condition, and they were at the MII stage of maturation (at this stage, the corona and cumulus layers are well expanded, zona pellucida is distinct, ooplasm is clear, and the first polar body is visualized).

The exclusion criteria were as follows: (1) females aged less than 20 years or older than 38 years; (2) the number of oocytes less than 6 and more than 7; (3) women with oocytes in different conditions; (4) ovarian problems or endometriosis; (5) males aged less than 20 years or more than 40 years; (6) sperm count less than 5×10^6/mL and more than 14×10^6/mL; and (7) sperm motility rate of more than 30% with any abnormalities such as leukocytospermia.

3.1. Studied Groups

The couples were randomly divided into three groups using the simple random sampling method (the table of random numbers) as follows: 1- control group (n = 28), 2- HOST group (n = 28) and (3) HA group (n = 24). The grouping was based on the sperm selection method for ICSI (Figure 1).

For the control group, sperm selection was performed on the basis of motility and morphology (routine method), for the HOST group, it was done on the basis of the HOST pattern, and for the HA group, it was on the basis of the binding of HA by using the PICSI (Origio) dishes.

3.2. Oocyte Collection

Ovulation induction protocols were carried out according to a previous study (20). Follicles 18 mm in diameter were collected by a transvaginal needle guided by ultrasound and were sent to a laboratory for ICSI.

3.3. Semen Analysis

For insemination, concurrent with oocyte collection, semen samples were obtained through masturbation after three to four days of sexual avoidance. Sperm counting was performed using the Neubauer chamber. For sperm morphology assessment, semen smear was prepared and slides

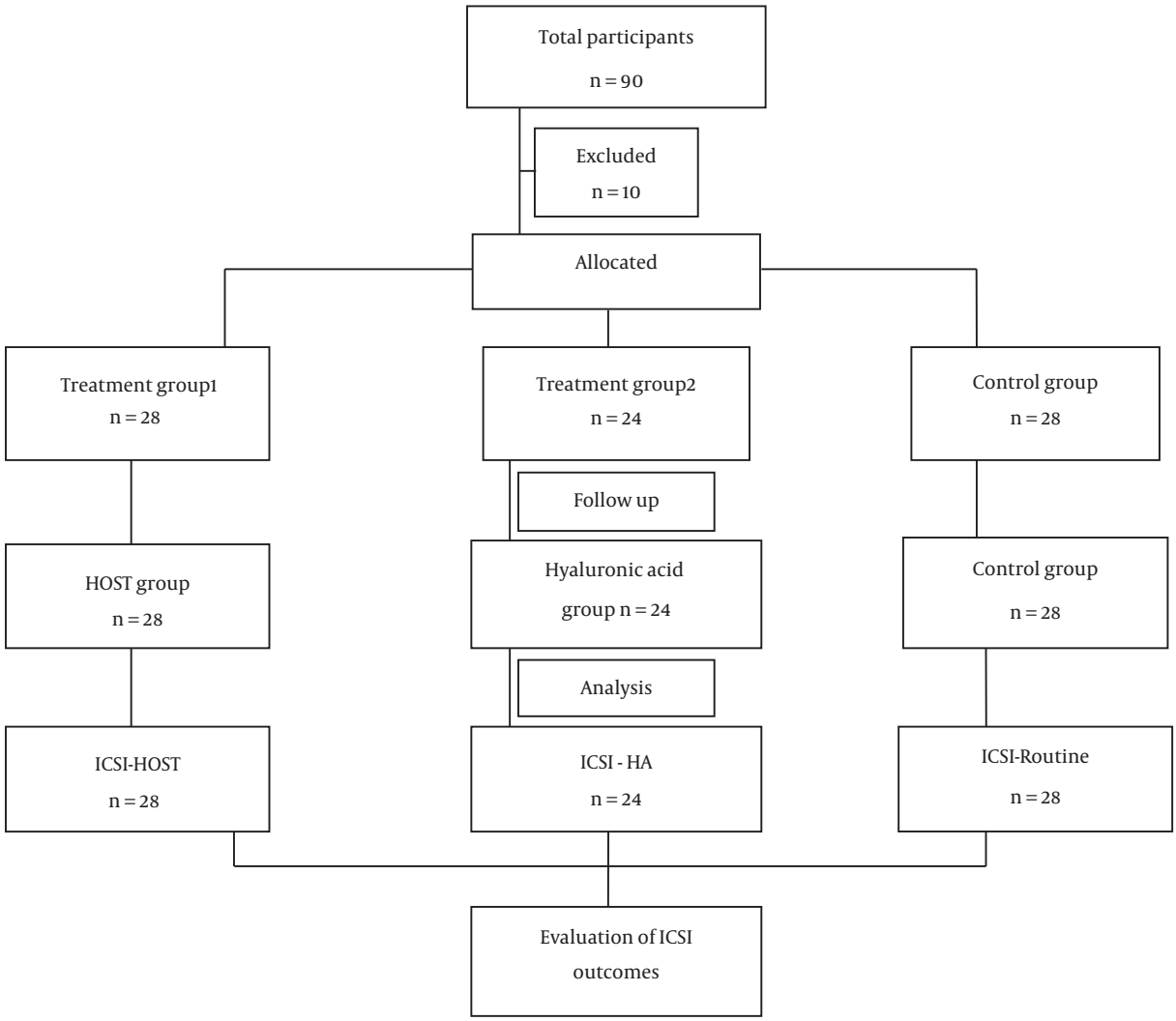

Figure 1. Flow chart of participant patients

were stained by using the Papanicolaou technique. Then, the samples were analyzed based on the World Health Organization (WHO) criteria (21). After analyzing the semen samples of infertile men with regards to the factors listed in our research, each sample was divided into two aliquots: One aliquot of the semen sample was treated via the routine sperm preparation technique for ICSI and another was applied for the assessment of DNA fragmentation by SCD test.

3.4. Density Gradient Centrifugation (Sperm Preparation)

For density gradient centrifugation, the 80/40 gradients (PureCeption™ Sperm Washing Medium, SAGE Media, USA) were prepared in a 14 mL tube. Then, 2 mL of semen was added and centrifugation was performed for 20 minutes at 200 g. The supernatant was discarded, and the pellet was kept undisturbed. The pellet was washed (200 g, 7 min) in 1 mL of the sperm preparation medium. The supernatant was again removed and the last pellet was coated with 0.5 mL of the sperm preparation medium. Afterward, the sample was incubated at 37°C for 30 minutes. Then, the upper layer was separated and used for ICSI (22).

3.5. Intracytoplasmic Sperm Injection

After retrieval, oocytes decumulation was carried out by using Hyase (Origio) and gentle pipetting. Then, the MII oocytes with the same condition were collected from each case to be inseminated with the sperm.

In the control group, after preparation, sperms were placed into polyvinylpyrrolidone (PVP) (Origio) for ICSI on the basis of morphology and motility. In the HOST group, sperms were put into a hypo-osmotic medium for 5 min at 37°C, and sperm selection was performed on the basis of the HOST pattern and according to the grading of HOST, through which the best sperm (grades d and e) was selected for insemination (18).

In the method of sperm binding to HA, PICSI® (Origio) was used according to the manufacturer's instructions that were as follows. First, 10 μL of the semen preparation medium was added to an HA drop on the PICSI® dish, which was then rehydrated for 5 to 10 min. Thereafter, the diluted sperm suspension was added and motile sperms and those connected to the drop were selected for oocyte insemination under an inverted microscope (10).

3.6. Evaluation of Sperm DNA Fragmentation

SDF assay kit (Avicenna) was utilized for the evaluation of DNA fragmentation according to the kit's protocol. Finally, 300 sperms were assessed manually on slides for the size of halo and dispersion pattern as characterized by Fernandez et al. The test results were categorized into nuclei with large, medium and small size halos and no halos (23). On the basis of the size of the halo around the head of the sperm, the SDF was determined. The nuclei with large- to medium-sized halos denoted a sperm with non-fragmented DNA, and nuclei with small-sized and zero halos were expressed as sperms with fragmented DNA. On the basis of the rate of SDF, DNA fragmentation was divided into three levels: 1-with less than 15% damage (good level), 2-between 15% and 30% damage (medium level) and 3-more than 30% damage (abnormal level) (Figure 2).

3.7. Assessment of Fertilization, Embryo Quality, and Embryo Development

Fertilization was evaluated based on the number of survived MII oocytes 16 to 20 hours after insemination by observing two pronuclei. Embryo development rate (EDR) was calculated according to the hypothesis of the normal growth of embryo at the 2-cell stage, 4-cell stage and 8-cell stage at 33.6, 45.5 and 56.4 hours after fertilization, respectively (8).

The quality of embryos was evaluated, and they were expressed as the two-cell, four-cell and eight-cell embryos. Embryo quality was evaluated by the Society for Assisted Reproduction Technology (SART) scoring system according to the symmetry of blastomere size and the quality and rate of cytoplasm fragments (24). On the third day after fertilization, three embryos were transferred to the mother's uterus. The implantation rate (IR) was determined on the basis of the observation of the gestational sac using ultrasound and human chorionic gonadotropin tests after 4 - 5 weeks (25).

3.8. Statistical Analysis

For data gathering, we used Intention-to-treat analysis, and for data analysis, one-way analysis of variance (ANOVA) for parametric variables and Kruskal-Wallis test for nonparametric variables were used. In addition, the Kolmogorov-Smirnov test was run for the assessment of the normal distribution of the data using SPSS Statistics for Windows, version 16.0 (SPSS Inc., Chicago, Ill., USA). A P value less than 0.05 was considered significant.

4. Results

With regards to the exclusion and inclusion criteria, 10 couples were excluded and 80 couples were included. The semen analysis reports and clinical characteristics of the 80 couples who were divided into the control, HOST and HA groups are shown in Table 1.

In this study, SDF was assessed by using the SCD test. According to the results, in 27 (33.7%) cases SDF was 15% to 30% and in 53 (66.3%) cases SDF was more than 30%. These cases were distributed in all the studied groups as follows; in the control group, in 37% of the cases, SDF was 15% to 30%, and in remaining 63% was more than 30%. In the HOST group, in 33.4% of the cases, SDF was 15% to 30%, and in the others was more than 30%. In the HA group, in 29.6% of the cases, SDF was 15% to 30%, while, it was more than 30% in the others. Therefore, no significant differences were observed among the studied groups in SDF, which was relatively equal in all the three groups (P = 0.959; Table 2).

Fertilization rate was evaluated according to the number of oocytes. Out of the 177, 81 and 149 oocytes in the control, HOST and HA groups, 142 (80%), 65(78%) and 114 (76%) oocytes were fertilized, respectively. The fertilization rate was higher in the control group in comparison with the other groups. However, this difference was not significant (P = 0.274; Table 2).

Embryo development was evaluated based on the cleavage stage and blastomere number on the third day after fertilization. The results indicated that the rate of the four-cell stage of embryonic development in the control group was 40%, the rate of the four-cell stage in the HOST group was 35% and the rate of the four-cell stage in the HA group was 33%. Moreover, the rates of the eight-cell stage of embryonic development in all the three groups were 64%, 67% and 67% in the control, HOST and HA groups, respectively. According to the results, the ED rates in the HOST and HA groups were higher than that in the control group.

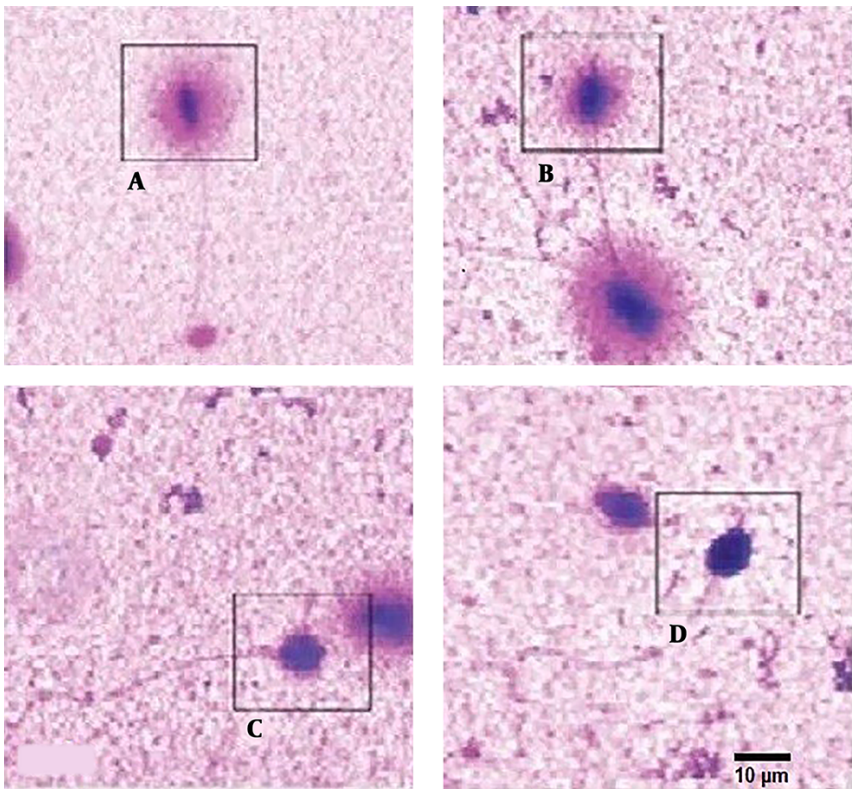

Figure 2. Sperm Chromatin Dispersion test. A, nucleus with a large-sized halo; B, nucleus with a medium-sized halo; C, nucleus with a small-sized halo; and D, nucleus without a halo (Diff-Quik)

Table 1. Semen Analysis and Clinical Characteristics of Couples Who Were Included in the Control, HOST and HA groups

Parameter	Control	Mean ± SD	HOST	Mean ± SD	Hyaluronic	Mean ± SD
Sperm concentration $\times 10^6$	10 - 14	12.51 ± 1.64	7 - 14	11.33 ± 2.23	5 - 14	10.92 ± 2.76
Sperm motility, %	15 - 22	18.71 ± 2.30	15 - 25	19.46 ± 3.14	15 - 28	20.87 ± 3.72
Normal morphology, %	17 - 42	27.25 ± 8.69	16 - 40	29.57 ± 7.65	14 - 38	25.04 ± 7.33
Female age, y	25 - 35	30.14 ± 3.01	27 - 37	30.53 ± 3.50	20 - 36	29.76 ± 3.95
Male age, y	30 - 40	35.37 ± 3.07	28 - 38	32.46 ± 3.14	25 - 39	31.00 ± 4.08
Duration of infertility, y	2 - 10	5.85 ± 2.69	5 - 7	7.35 ± 1.70	3 - 8	5.41 ± 1.71

Abbreviations: HA, hyaluronic acid; HOST, hypo-osmotic swelling test.

However, the difference between the groups was not significant (P = 0.082; Table 2).

In terms of the quality of four-cell embryos, in the control, HOST and HA groups, 56%, 62% and 60% were good grade embryos. These results showed an insignificant difference between the groups with respect to the qualities of four-cell embryos (P = 0.645; Table 2).

With regards to the qualities of the eight-cell embryos, 54%, 83% and 66% in the control, HOST and HA groups were good grade embryos. These results showed a significant difference between the groups in terms of the good grade of the eight-cell embryos (P = 0.001; Table 2). With respect to the parameter of the good grade of eight-cell embryos, the control group compared to the HOST group (P = 0.001), the HOST group versus the HA group (P = 0.029) and the control group versus the HA group (P = 0.044) were significantly different. In the parameter of the fair grade of the eight-cell embryo quality, the control group versus the HOST group (P = 0.002), the HOST group versus the HA group (P = 0.032) and the control group versus the HA

Table 2. The Comparison Between the Sperm DNA Fragmentation (SDF) Rate, Fertilization Rate, Early Embryo Development, Embryo Quality, and the Implantation Rate in the Studied Groups

Parameters	Control Group	HOST Group	HA Group	P Value
Fertilization rate	0.80 ± 0.108	0.78 ± 0.160	0.76 ± 0.131	0.274
ED of four-cell on day 3	0.40 ± 0.102	0.35 ± 0.201	0.33 ± 0.114	0.098
ED of eight-cell day 3	0.64 ± 0.113	0.67 ± 0.229	0.67 ± 0.114	0.082
EQ of four-cell (good grade)	0.56 ± 0.219	0.62 ± 0.498	0.60 ± 0.364	0.645
EQ of four-cell (fair grade)	0.44 ± 0.219	0.38 ± 0.498	0.40 ± 0.364	0.645
EQ of eight-cell (good grade)	0.54 ± 0.228	0.83 ± 0.389	0.66 ± 0.181	0.001[a]
EQ of eight-cell (fair grade)	0.46 ± 0.228	0.21 ± 0.312	0.34 ± 0.181	0.002[b]
Sperm DNA fragmentation (SDF) rate between 15% and 30%	0.357 ± 0.488	0.33 ± 0.480	0.32 ± 0.476	0.959
SDF rate more than 30%	0.64 ± 0.488	0.66 ± 0.480	0.68 ± 0.476	0.959
Implantation rate	0.21 ± 0.418	0.32 ± 0.487	0.35 ± 0.510	0.021[a]

Abbreviations: ED, embryo development; EQ, embryo quality.
[a] P < 0.05.
[b] P < 0.01.

group (P = 0.044) were significantly different (Table 2).

Our results related to the IR revealed that in the control group the IR was 21%, in the HOST group it was 32%, and in the HA group IR was 35%. The IR was dramatically less in the control group in comparison with the HA group (P = 0.021; Table 2). However, no significant difference was noted between the HOST and HA groups.

5. Discussion

In general, an increase in the incidence of male infertility has been observed, and many factors are involved in this condition, including exposure to chemicals and hazardous environmental factors such as overheating, water and air pollution and stress (26). These conditions lead to an increased production of reactive oxygen species, which in turn lead to sperm DNA damage. DNA contains genetic information and serves a role in gene replication and transcription, which eventually results in reproduction and growth (27).

DNA fragmentation is one of the important causes of male infertility (28). Also, it has been demonstrated that the level of SDF could affect pregnancy success rate (29, 30). Technological advancements have provided a solution for male infertility through the administration of ICSI (31). However, the major drawback of this method is the process of sperm selection. Because of this, the use of ICSI would remain controversial until the discovery of a proper and safe method, which would be similar to the natural sperm selection process.

Nevertheless, it has been reported that annually more than 30,000 ICSIs have been conducted in the United States, and this method is used in about 70% of IVF cases in some infertility centers in order to increase the fertility rate (32). Concerns regarding sperm selection are due to the increased risk of aneuploidy, sperm apoptosis, and SDF (8). Therefore, the aim of sperm selection for assisted reproduction is to select natural and healthy sperm, which can facilitate the process of fertilization and lead to improved clinical outcomes.

In our study, the evaluation of SDF levels revealed that DNA damage was high in males with oligoasthenozoospermia, which is in line with the previous studies that have demonstrated that infertile men have high levels of DNA damage (6).

Due to the fact that sperm with normal morphology and motility can also have DNA fragmentation (33), it is possible for sperms with fragmented DNA to be fertilized, especially in oligoasthenozoospermic men. Thus, the results of the ICSI technique in oligoasthenozoospermic men depend on sperm quality and could effectively influence fertilization success, embryo quality, embryo development, and IR.

As indicated in the results of our study, no difference was seen between the studied groups in fertilization results caused by the ICSI method despite the high level of SDF. However, the results obtained by Pregl Breznik et al. revealed that the level of fragmented DNA could affect the fertilization rate in the IVF method (20).

Literature shows contradictory findings of the relationship between the level of SDF and fertilization after ICSI. A previous study showed a negative relationship between the high level of SDF and fertilization after ICSI (34), whereas other studies did not show any effect on fertil-

ization rate after ICSI, but they showed a negative effect on embryo quality and pregnancy rate (35). These results could be due to the fact that in the ICSI method, the natural process of fertilization is eliminated and the sperms are injected into the oocyte despite having chromosomal defects, low motility, weak zona pellucida binding, and incomplete acrosome reaction (36). The results might be due to treat the damaged DNA during fertilization by the oocyte. The oocyte despite having the ability to improve DNA damage, but it depends on the content of the SDF that might involve one or both DNA chains. Although one damaged DNA chain could be repaired during fertilization by the oocyte, fertilization and the natural development of the embryo are difficult in cases where two DNA chains are damaged (27). Therefore, the reason for the high fertilization rate in the studied groups, especially in the control group, may be justified here.

In addition, the control group had lower level of embryo quality and development compared with the other groups. This difference might be due to the fact that in the early stages of development, the paternal genome is activated after the four-cell stage, which was formerly controlled by the mRNA inherited from the maternal genome (37, 38). Therefore, SDF might have an impact on blastocyst development after the activation of the paternal genome. Consequently, the difference could be observed at this stage, and our results are in congruence with this theory. Previous studies have suggested that to obtain better results, sperm selection should be carried out based on sperm performance and not with respect to morphology and motility (39). In so doing, the chance of embryo quality and development is increased.

Recently, it was found that sperm membrane integrity dysfunction can be identified by the HOST test, and the integrity of sperm membrane may reflect the degree of DNA integration (40). Research results showed a relationship between DNA integrity and different degrees of sperm tail curvature due to exposure to the hypo-osmotic condition (15, 16). In this method, according to evaluations, grade D and E sperms were associated with minimal DNA damage and protamine deficiency, and grade g sperm was related to a higher frequency of sperm abnormalities and DNA damage. The sperm selection with healthy DNA based on HOST grading is feasible and suggested to use this procedure for improving ICSI outcome. Also, it is useful for identifying viable and healthy sperms in cases where sperms are completely inactive such as Kartagener's syndrome for ICSI (39).

Our results are in agreement with the abovementioned findings and showed that the HOST method could be more effective compared to the HA method in the selection of healthy sperm and improving the outcomes of ICSI. In addition, the HOST method is an easy, fast, and inexpensive approach for use in assisted reproduction technology laboratory.

Furthermore, the results of our previous study, where sperm selection with the conventional method was performed, showed that ICSI outcomes in oligoasthenozoospermic men who had SDF between 15% and 30% were better in comparison with the oligoasthenozoospermic men with an SDF more than 30%. In this study also we noted that better ICSI outcomes can be derived from an appropriate method of sperm selection in oligoasthenozoospermic men.

A dramatic increase in the IR was seen in the HA and HOST groups in this study, which confirmed the positive impact of selection of healthy sperm on IR after ICSI. Consistent with the literature, our findings indicated that oocyte fertilization with sperm containing damaged DNA might increase the risk of pregnancy failure (20). In sum, the HOST method is as beneficial as the HA binding technique in oligoasthenozoospermic men in terms of increasing the IR.

5.1. Conclusions

In oligoasthenozoospermic men who underwent ICSI, sperm selection through the HOST and HA techniques led to improved results compared with the routine method, in which sperm is selected on basis of its motility and morphology. The comparison of embryo quality and development between the HOST and HA groups yielded similar results. However, a slight increase was observed in the HOST group despite the fact that the HOST method was less costly and more easily available and efficient compared with the HA method. Therefore, the HOST method is recommended for further investigation and use for sperm selection in the ICSI technique for oligoasthenozoospermic men.

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Footnotes

Conflict of Interests: There was not any conflict of interest.

Ethical Considerations: All the participants completed the registration forms for participation in the study. They were ensured of the confidentiality of the data. The study

was accepted by the Ethics Committee of Mashhad University of Medical Sciences, Khorasan Razavi province, Iran (code number IR.mums.fm.REC.1394.320) and registration ID in IRCT: IRCT2017090336039N1.

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References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. 2015;**13**:37. doi: 10.1186/s12958-015-0032-1. [PubMed: 25928197]. [PubMed Central: PMC4424520].
2. Lamothe S, Kerlan V, Christin-Maitre S. [Semen quality and fertility: The role of the environment and health]. *Ann Endocrinol (Paris)*. 2018;**79 Suppl 1**:S1–9. French. doi: 10.1016/S0003-4266(18)31233-2. [PubMed: 30213300].
3. Song SH, Chiba K, Ramasamy R, Lamb DJ. Recent advances in the genetics of testicular failure. *Asian J Androl*. 2016;**18**(3):350–5. doi: 10.4103/1008-682X.178857. [PubMed: 27048782]. [PubMed Central: PMC4854078].
4. Brezina PR, Zhao Y. The ethical, legal, and social issues impacted by modern assisted reproductive technologies. *Obstet Gynecol Int*. 2012;**2012**:686253. doi: 10.1155/2012/686253. [PubMed: 22272208]. [PubMed Central: PMC3261493].
5. Hoorsan H, Mirmiran P, Chaichian S, Moradi Y, Hoorsan R, Jesmi F. Congenital malformations in infants of mothers undergoing assisted reproductive technologies: A systematic review and meta-analysis study. *J Prev Med Public Health*. 2017;**50**(6):347–60. doi: 10.3961/jpmph.16.122. [PubMed: 29207452]. [PubMed Central: PMC5717326].
6. Avendano C, Oehninger S. DNA fragmentation in morphologically normal spermatozoa: How much should we be concerned in the ICSI era? *J Androl*. 2011;**32**(4):356–63. doi: 10.2164/jandrol.110.012005. [PubMed: 21088229].
7. Aitken RJ, Koopman P, Lewis SEM. Male germs of concern. *Nature*. 2004;**432**:2–6.
8. Parmegiani L, Cognigni GE, Bernardi S, Troilo E, Ciampaglia W, Filicori M. "Physiologic ICSI": Hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. *Fertil Steril*. 2010;**93**(2):598–604. doi: 10.1016/j.fertnstert.2009.03.033. [PubMed: 19393999].
9. Menezo Y, Clement P, Amar E. Evaluation of sperm DNA structure, fragmentation and decondensation: An essential tool in the assessment of male infertility. *Transl Androl Urol*. 2017;**6**(Suppl 4):S553–6. doi: 10.21037/tau.2017.03.11. [PubMed: 29082177]. [PubMed Central: PMC5643722].
10. Erberelli RF, Salgado RM, Pereira DH, Wolff P. Hyaluronan-binding system for sperm selection enhances pregnancy rates in ICSI cycles associated with male factor infertility. *JBRA Assist Reprod*. 2017;**21**(1):2–6. doi: 10.5935/1518-0557.20170002. [PubMed: 28333023]. [PubMed Central: PMC5365191].
11. Nasr Esfahani MH, Deemeh MR, Tavalaee M, Sekhavati MH, Gourabi H. Zeta sperm selection improves pregnancy rate and alters sex ratio in male factor infertility patients: A double-blind, randomized clinical trial. *Int J Fertil Steril*. 2016;**10**(2):253–60. [PubMed: 27441060]. [PubMed Central: PMC4948079].
12. Beck-Fruchter R, Shalev E, Weiss A. Clinical benefit using sperm hyaluronic acid binding technique in ICSI cycles: A systematic review and meta-analysis. *Reprod Biomed Online*. 2016;**32**(3):286–98. doi: 10.1016/j.rbmo.2015.12.001. [PubMed: 26776822].
13. Simopoulou M, Gkoles L, Bakas P, Giannelou P, Kalampokas T, Pantos K, et al. Improving ICSI: A review from the spermatozoon perspective. *Syst Biol Reprod Med*. 2016;**62**(6):359–71. doi: 10.1080/19396368.2016.1229365. [PubMed: 27646677].
14. Rossato M, Di Virgilio F, Foresta C. Involvement of osmo-sensitive calcium influx in human sperm activation. *Mol Hum Reprod*. 1996;**2**(12):903–9. [PubMed: 9237233].
15. Stanger JD, Vo L, Yovich JL, Almahbobi G. Hypo-osmotic swelling test identifies individual spermatozoa with minimal DNA fragmentation. *Reprod Biomed Online*. 2010;**21**(4):474–84. doi: 10.1016/j.rbmo.2010.06.026. [PubMed: 20833108].
16. Bassiri F, Tavalaee M, Shiravi AH, Mansouri S, Nasr-Esfahani MH. Is there an association between HOST grades and sperm quality? *Hum Reprod*. 2012;**27**(8):2277–84. doi: 10.1093/humrep/des155. [PubMed: 22595979].
17. Rouen A, Carlier L, Heide S, Egloff M, Marzin P, Ader F, et al. Potential selection of genetically balanced spermatozoa based on the hypo-osmotic swelling test in chromosomal rearrangement carriers. *Reprod Biomed Online*. 2017;**35**(4):372–8. doi: 10.1016/j.rbmo.2017.06.017. [PubMed: 28711302].
18. Charehjooy N, Najafi MH, Tavalaee M, Deemeh MR, Azadi L, Shiravi AH, et al. Selection of sperm based on hypo-osmotic swelling may improve ICSI outcome: A preliminary prospective clinical trial. *Int J Fertil Steril*. 2014;**8**(1):21–8. [PubMed: 24695913]. [PubMed Central: PMC3973167].
19. Hox J, Roberts JK. *Handbook of advanced multilevel analysis*. 1st ed. New York: Routledge; 2011. p. 266–8.
20. Pregl Breznik B, Kovacic B, Vlaisavljevic V. Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? *Fertil Steril*. 2013;**99**(5):1233–41. doi: 10.1016/j.fertnstert.2012.11.048. [PubMed: 23290739].
21. World Health Organisation. *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. Cambridge University Press; 1999.
22. Jayaraman V, Upadhya D, Narayan PK, Adiga SK. Sperm processing by swim-up and density gradient is effective in elimination of sperm with DNA damage. *J Assist Reprod Genet*. 2012;**29**(6):557–63. doi: 10.1007/s10815-012-9742-x. [PubMed: 22411295]. [PubMed Central: PMC3370043].
23. Fernandez JL, Muriel L, Goyanes V, Segrelles E, Gosalvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. *Fertil Steril*. 2005;**84**(4):833–42. doi: 10.1016/j.fertnstert.2004.11.089. [PubMed: 16213830].
24. Heitmann RJ, Hill MJ, Richter KS, DeCherney AH, Widra EA. The simplified SART embryo scoring system is highly correlated to implantation and live birth in single blastocyst transfers. *J Assist Reprod Genet*. 2013;**30**(4):563–7. doi: 10.1007/s10815-013-9932-1. [PubMed: 23443889]. [PubMed Central: PMC3644132].
25. Parmegiani L, Cognigni GE, Filicori M. New advances in intracytoplasmic sperm injection (ICSI). *Advances in embryo transfer*. InTech; 2012.
26. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. 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**(2):120–7. doi: 10.1016/j.rbmo.2014.10.018. [PubMed: 25530036].
27. Sakkas D, Alvarez JG. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril*. 2010;**93**(4):1027–36. doi: 10.1016/j.fertnstert.2009.10.046. [PubMed: 20080235].
28. Zeqiraj A, Beadini S, Beadini N, Aliu H, Gashi Z, Elezaj S, et al. Male infertility and sperm DNA fragmentation. *Open Access Maced J Med Sci*. 2018;**6**(8):1342–5. doi: 10.3889/oamjms.2018.311. [PubMed: 30159053]. [PubMed Central: PMC6108823].
29. Aydos OS, Yukselten Y, Kaplan F, Sunguroglu A, Aydos K. Analysis of the correlation between sperm DNA integrity and conven-

tional semen parameters in infertile men. *Turk J Urol*. 2015;**41**(4):191–7. doi: 10.5152/tud.2015.98475. [PubMed: 26623148]. [PubMed Central: PMC4621147].
30. Cho CL, Agarwal A. Role of sperm DNA fragmentation in male factor infertility: A systematic review. *Arab J Urol*. 2018;**16**(1):21–34. doi: 10.1016/j.aju.2017.11.002. [PubMed: 29713533]. [PubMed Central: PMC5922225].
31. Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian J Androl*. 2017;**19**(1):80–90. doi: 10.4103/1008-682X.182822. [PubMed: 27345006]. [PubMed Central: PMC5227680].
32. Palermo GD, O'Neill CL, Chow S, Cheung S, Parrella A, Pereira N, et al. Intracytoplasmic sperm injection: State of the art in humans. *Reproduction*. 2017;**154**(6):F93–F110. doi: 10.1530/REP-17-0374. [PubMed: 29158352]. [PubMed Central: PMC5719728].
33. Alvarez Sedo C, Bilinski M, Lorenzi D, Uriondo H, Noblia F, Longobucco V, et al. Effect of sperm DNA fragmentation on embryo development: Clinical and biological aspects. *JBRA Assist Reprod*. 2017;**21**(4):343–50. doi: 10.5935/1518-0557.20170061. [PubMed: 29116706]. [PubMed Central: PMC5714603].
34. Seli E, Gardner DK, Schoolcraft WB, Moffatt O, Sakkas D. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. *Fertil Steril*. 2004;**82**(2):378–83. doi: 10.1016/j.fertnstert.2003.12.039. [PubMed: 15302287].
35. Sakkas D. The use of blastocyst culture to avoid inheritance of an abnormal paternal genome after ICSI. *Hum Reprod*. 1999;**14**(1):4–5. [PubMed: 10374083].
36. Wang C, Feng G, Zhang B, Shu J, Zhou H, Gan X, et al. Influence of the insemination method on the outcomes of elective blastocyst culture. *Clin Exp Reprod Med*. 2017;**44**(2):85–9. doi: 10.5653/cerm.2017.44.2.85. [PubMed: 28795047]. [PubMed Central: PMC5545224].
37. Rappa KL, Rodriguez HF, Hakkarainen GC, Anchan RM, Mutter GL, Asghar W. Sperm processing for advanced reproductive technologies: Where are we today? *Biotechnol Adv*. 2016;**34**(5):578–87. doi: 10.1016/j.biotechadv.2016.01.007. [PubMed: 26845061].
38. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, et al. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod*. 1998;**59**(5):1037–46. [PubMed: 9780307].
39. Westlander G, Barry M, Petrucco O, Norman R. Different fertilization rates between immotile testicular spermatozoa and immotile ejaculated spermatozoa for ICSI in men with Kartagener's syndrome: Case reports. *Hum Reprod*. 2003;**18**(6):1286–8. [PubMed: 12773460].
40. Herbemont C, Sifer C. How to select the spermatozoon for intracytoplasmic sperm injection in 2015? *Minerva Ginecol*. 2015;**67**(2):185–93. [PubMed: 25581325].