BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2023. Vol. **20**(3), p. 983-992

Association between Anti-Sperm Antibody and Intra-cytoplasmic Sperm Injection Outcomes Among Male Infertility Patients

Haider Rafea Chiflawy Alkhafaji[1]*, Sahib Yahiya Hassan[2] and Saaduldeen Gali Al-Esawi[2]

[1]Department of Urology-Clinical Embryology, Faculty of Medicine, University of Kufa, Najaf, Iraq.
[2]Department of Urology and Infertility, Faculty of Medicine, University of Kufa, Iraq.

https://dx.doi.org/10.13005/bbra/3149

(Received: 16 July 2023; accepted: 09 August 2023)

Infertility, a clinical and public concern, affects both social life and the health system. Sperm abnormalities which include (Oligozoospermia), (teratozoospermia) or abnormalities related to sperm motility are essential factors in male infertility. Anti-sperm antibodies (ASA) are the leading cause of immune infertility in men (autoimmune disease). Intra-cytoplasmic Sperm Injection (ICSI) is specially designed to manage severe cases of male-factor infertility. this study aimed to determine the effect of ASA on ICSI outcomes among Normospermia and (Oligozoospermia, Asthenozoospermia and Teratozoospermia). This is a cross-sectional study performed at Fertility Center, Najaf-Iraq between Jan to June 2023, it included 50 couples who suffered from a minimum of 12 months of primary fertility who had attended the Fertility Center. They were divided into four groups (Normospermia, Oligospermia, Teratozoospermia, and Asthenospermia) according to their seminal fluid analysis (SFA). Sperms were collected by ICSA, immobilized, and processed. The oocytes were collected, injected, and prepared for fertilization, after that fertilization took place. Finally, pregnancy was examined in each female partner. There was no significant difference in the age of the four groups, there was no relationship between ASA and Sperm concentration, and there was no relationship between ASA and Normal Sperm Morphology, fertilization , & pregnancy rates, in addition to other ICSI outcomes, were similar among the four groups. Pregnancy rates were higher in the normospermia group than the other three groups and ICSI overcomed the presence of ASA in the semen plasma.

Keywords: Anti-Sperm Antibody; Intra-cytoplasmic Sperm Injection; Infertility.

Infertility is a clinical and public concern as it affects both social life and the health system [1]. According to the World Health Organization 2020, infertility is "a condition of the reproductive system characterized as a couple's failure to conceive after twelve months of unprotected sexual intercourse in the fertile phase of the menstrual cycle among women younger than 35 and the failure to conceive after six months of regular unprotected sexual intercourse in women aged 35 or older" [2]. Problems with sperm production, low sperm counts, abnormal sperm morphology, or sperm motility

*Corresponding author E-mail: haiderr.alkhafaji@student.uokufa.edu.iq

This is an Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).
Published by Oriental Scientific Publishing Company © 2023

are the most common causes of male infertility [3]. Despite its limitations, semen analysis remains the primary method for determining the cause of male infertility [4]. It is performed with high standards to evaluate the ejaculate's descriptive parameters [5,6]. Despite the useful information revealed by the initial evaluation of the semen analysis assay, it is not confirmed as a reliable test of fertility.

Sperm abnormalities are an essential factor in male infertility. Low sperm count is known as (Oligozoospermia: <15 million sperms/ mL)[7]. Motility abnormalities in sperm are a common cause of infertility, and a typical semen assay should show at least 50% grade A and B, progressively motile spermatozoa [8]. Failure to fertilize is indicated by low motility that persists over time. Finally, irregularities in the shape and structure of the sperm (teratozoospermia) [9]. The coexistence of these abnormalities is called Oligoasthenoteratozoospermia (OAT syndrome).

Anti-sperm antibodies (ASA) are the leading cause of immune infertility in men (autoimmune disease). Significant levels of anti-sperm antibodies are detected in the semen of 5% to 15% of infertile men, and only 1% to 2% of fertile men. Anti-sperm antibodies are found in men's serum, seminal plasma, and bind to sperm. Even though the mechanism of anti-sperm antibodies lead to infertility in men is vague, various studies have concluded that there is a negative impact of anti-sperm antibodies on sperm concentration, motility, and liquefaction [10].

Intra-cytoplasmic Sperm Injection (ICSI) is a specially designed form of in Vitro Fertilization (IVF) used mainly to manage severe cases of male-factor infertility, ICSI involves the injection of a single sperm that has been surgically retrieved from the epididymis or testis into a mature egg [11]. Despite the fact that high success rates for fertilization in ICSI treatments suggest hazards for infants, there is only a minor increase in de novo chromosomal abnormalities. However, the rate of serious congenital malformations is comparable for IVF and ICSI (3 to 4 percent). According to the Bayley scale, the developmental outcome of IVF and ICSI is comparable around two years of age [12].

This study aimed to investigate the impact of Anti-Sperm Antibodies on ICSI outcomes for male infertility patients and to measure the relationship between them.

MATERIALS AND METHODS

The current study design is a cross-sectional study performed at Fertility Center, Najaf-Iraq. It was held from Jan to June 2023.

This study obtained ethical approval from the internal ethical committee of the Urology-Clinical Embryology department/Faculty of Medicine/University of Kufa and the Health Directorate in Najaf province.

It included 50 couples who suffered from a minimum of 12 months of primary fertility with regular unprotected sexual intercourse and infertility factors related to males who had attended the Fertility Centre requesting fertility treatment.

Gynaecological examination and assessment were done for the participants' female partners, while the urologists examined and assessed the male subjects.

Inclusion Criteria was:Patients with Normospermia or mild (Oligospermia ,Asthenospermia ,Teratozoospermia)

Exclusion Criteria for Male was: Severe condition of (Oligozoospermia , Asthenozoospermia (obstructive) , Teratozoospermia)

Inclusion Criteria for the Female was:Healthy female, Young females 21- 43 years old, -Healthy weight

Exclusion Criteria for the Female was: Extreme age older than 43 & younger than 21 years old, Overweight female partners, Underweight female, Female with polycystic ovarian syndrome (PCOS), Female partners with Ovarian hyper-stimulation syndrome (OHSS)

Data and Sample collection

The participants received oral instructions regarding the method of sample collection and preparation advice, such as abstinence from sexual activity for a minimum of two to seven days. The fresh ejaculate was collected from the participants in a sterile container (wide-mouthed) by masturbation in a laboratory room isolated for privacy and ethical purposes. Each container has the participant's identification details, such as name, age, and the sample collection time, as they were instructed that the sample must be complete. Semen from each participant included in the study was obtained from the participants' ejaculated samples.

Sample collection and storage

Semen samples after conventional examination were centrifuged for 10 min at 1000 rpm within 30 min of collection, and supernatants were collected and kept in the refrigerator at–20°C for collective measurement of the whole samples by the enzyme-linked immunosorbent assay (ELISA) method later.

Detection of ASA in seminal plasma by ELISA technique

The ELISA plate manufactured by (Demieditec Diagnostics GmbH / Germany) is coated with anti-spermatozoa antibodies that recognize a mixture of spermatozoa proteins. To prevent liquid loss from evaporation, standards and samples are pipetted into the wells and then incubated in a humidified chamber. During this incubation, anti-spermatozoa antibodies bind to spermatozoa proteins, immobilizing them on a plate. After washing away any unattached compounds, the enzyme conjugate, a human antibody conjugated with Horse Radish Peroxidase (HRP), is applied to each well and incubated. After washing away any unbound antibody–enzyme reagent, Tetramethylbenzidine (TMB) substrate solution is applied to the wells. The color of the solution changes from blue to yellow based on the quantity of antibodies bound in the first stage. The color intensity is measured using a Human Reader HS machine ELISA reader at 450 nm wavelength. Normal values are 0–60 U/ml, while elevated (positive) values are >60 U/ml. The results were calculated after the standard curve was generated.

ICSI procedure

Ovulation induction was carried out using a combination of gonadotropin-releasing hormone agonist and human menopausal gonadotropin. Ovulation was triggered by human chorionic gonadotropin (HCG), when ~*3 follicles measured ~*18 mm in diameter and serum estradiol concentration was at least 1000 ng/L. Oocyte retrieval was carried out 36 h later, followed by oocyte assessment for maturity under an inverted microscope, then preparation for ICSI, which include removal of the cumulus and corona radiata cells (denudation). Every mature oocyte was injected with a single, living, immobilized spermatozoon, then fertilization was confirmed 16–18 h later by observation of two distinct pronuclei (2PN) and two polar bodies. The fertilization rate was calculated as the number of fertilized oocytes divided by the total number of mature oocytes for each couple Fertilized oocytes were observed for embryonic development on day 3 after injection, just prior to the transfer, and classified as previously described [13] into:

• Good quality grades A embryo (0–20% of the volume filled with anucleated fragments).

• Fair quality grade B embryos (20–50% anucleated fragments).

• Poor quality grade C embryos (>50% anucleated fragments).

Pregnancy examination

The transfer of the best available embryos from the first two grades. 14 days later, the serum human chorionic gonadotropin (HCG) level was examined to determine a biochemically positive pregnancy. In such instances, clinical pregnancy was ultrasonography confirmed 5–6 weeks after injection.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 22; an alpha value (P) of dH .05 was considered statistically significant. Some results were expressed by their mean ± SD. Chi-square test used to compare between categorical variables. one-way ANOVA used to compare between categorical variables with more than two sub groups.

RESULTS

The study included 50 couples; the male participants were classified into four groups (Normospermia, Oligospermia, Teratozoospermia, and Asthenospermia) according to their seminal fluid analysis SFA. The mean age of the Normo group was 38 ± 11 years, while their female partners' mean age was 32 ± 5 years old. The mean age of the Oligo group was 36 ± 4 years, while their female partners' mean age was 32 ± 5 years old. The mean age of the Astheno group was 40 ± 9 years, while their female partners' mean age was 31 ± 6 years old. The mean age of the Terato group was 35 ± 8 years, while their female partners' mean age was 33 ± 6 years old. There was no significant difference in the age of the four groups (P value= 0.6), as shown in Table 1.

Sociodemographic variables such as the participant's age, height, weight, infertility

duration, infertility type, testicular varicocele, smoking, wife's age, and abstinence period are represented in Table 1. There was no significant difference among the four groups for almost all of these variables as (P value<0.05), apart from being diagnosed with testicular varicocele (*P* value = 0.01) which is significant, Table 1.

Regarding sperm parameters variables among the four groups (Normo, Oligo, Astheno, Terato), there was a significant difference in Sperm concentration (*P* value = 0.006), Progressive sperm motility (*P* value <0.001), and Sperm normal morphology (*P* value <0.001) among the four groups, Table 1.

Pearson's correlation was performed to investigate the relationship between ASA and Sperm concentration in the studied samples (50 participants). It was found that there is no relationship between the two (P value = 0.3, r = 0.152), as shown in Table 3.

Pearson's rank correlation test was done to investigate the relationship between ASA and Progressive Sperm Motility in the studied samples (50 participants). It was found that there is no

Table 1. Demographic variables analysis & Sperm Parameters in Normo, Oligo, Astheno, and Terato samples of patients who underwent the ICSI program

Variable	Normo (N=21) 42%	Oligo (N=7) 14%	Astheno (N=7) 14%	Terato (N=15) 30%	*P* value
Age years	Mean ± SD 38 ± 11	Mean ± SD 36 ± 4	Mean ± SD 40 ± 9	Mean ± SD 35 ± 8	0.6
Height cm	176 ± 6	177 ± 6	178 ± 5	176 ± 6	0.8
Weight Kg	83 ± 11	91 ± 17	86 ± 10	89 ± 8	0.3
Infertility duration	5 ± 3	9 ± 7	7 ± 4	8 ± 5	0.4
Infertility type	Primary =10 Secondary =11	Primary = 4 Secondary = 3	Primary = 1 Secondary = 6	Primary = 11 Secondary = 4	0.07
Testicular varicocele	Yes = 6 No =15	Yes = 4 No = 3	Yes = 7 No = 0	Yes = 8 No = 7	0.01**
Smoking	Yes = 12 No = 9	Yes = 6 No = 1	Yes = 6 No = 1	Yes = 9 No = 6	0.3
Wife's age years	32 ± 5	32 ± 5	31 ± 6	33 ± 6	0.9
Abstinence period	3 ± 0.6	4 ± 0.8	3 ± 0	3 ± 0.4	0.2
Sperm concentration million/ml	59.8±24.5	27.6±28.2	46.6±31.7	32.7±21.8	0.006*
Progressive sperm motility %	57 ± 17	30 ± 33	14 ± 13	26 ± 15	<0.001*
Sperm normal morphology %	38 ± 20	28 ± 37	22 ± 11	4 ± 2	<0.001*

(P value ≤ 0.05 is significant for the one-way ANOVA test*. P value ≤ 0.05 is significant for the Chi-square test**.)

Table 2. Comparison of Anti-Sperm Antibody levels in the seminal fluid samples of Oligospermia, Asthenospermia, & Teratospermia patients compared to samples of Normospermia patient

Variable	Sperm classification	Number	Mean	*P* value
ASA	NormospermiaOligospermia	217	71.7155.7	0.2
ASA	NormospermiaAsthenospermia	217	71.762.3	0.8
ASA	NormospermiaTeratozoospermia	2115	71.747.7	0.5

P value ≤ 0.05 is significant for T-test*.

relationship between the two (P value = 0.1, r = -0.221), as shown in Table 3.

Pearson's rank correlation test was conducted to investigate the relationship between ASA and Normal Sperm Morphology in the studied samples (50 participants). Non-significant relationship between the two variables (P value = 0.3, r = 0.158), as shown in Table 3.

Table 4 illustrates the primary ICSI outcomes among the four groups included in the study according to sperm classification (Norm (n=21), Oligo (n=7), and Astheno (n=7), Terato (n=15)). ANOVA test revealed that there was no significant difference in the retrieved oocytes among the four groups (*P* value=0.3). Similarly, there was no significant difference in the MII oocytes for (Normo mean ± SD=8.9±5.6), (Oligo 5.9±2.5), (Astheno 8.3±6.8), and (Terato participants as (*P* value =0.6). Likewise, the ANOVA test was done, and there was no significant difference in the fertilization rate (*P* value =0.2), cleavage rate (*P* value =0.8), and 2PN (*P* value =0.6) among the four groups (Norm, Oligo, Astheno, Terato). A chi-square test revealed that there was no significant difference in the pregnancy ratio (*P* value =0.5). Participants with Normospermia samples who

Table 3. The relationship between Anti-Sperm Antibody & sperm parameters

First variable	Second Variable	r value	P value
ASA	Sperm concentration	0.152	0.3
ASA	Progressive sperm Motility	- 0.221	0.1
ASA	Normal Sperm Morphology	0.158	0.3

P value ≤ 0.05 is significant for Pearson's correlation test*.

Table 4. Comparison of Intra-Cytoplasmic Sperm Injection outcomes in Normo, Oligo, Astheno, &Terato patients

Variables	Normospermia N=21	Oligospermia N=7	Asthenospermia N=7	Teratozoospermia N=15	*P* value
Retrieved oocytes	11.5±6	6.3±2.4	10±9	9.5±6.8	0.3
Fertilization rate	72.9±19	88.6±18.6	86±13	78±21	0.2
Pregnancy Ratio	Pregnancy 61.9%	Pregnancy 57.1%	Pregnancy 28.6%	Pregnancy 46.66%	0.5

P value ≤ 0.05 is significant for the one-way ANOVA test*.
P value ≤ 0.05 is significant for the Chi-square test**.

Table 5. A comparison in sperm parameters according to normal and abnormal distribution of ASA among the participants

Sperm parameters	Anti-Sperm Antibodies (ASA) ≤ 60 U/ml 34 participant (68%)	≥ 60 U/ml 16 participant (32%)	P value
Sperm concentration million/ml	43.7±28.5	48.8±27.4	0.6
Progressive sperm motility %	40.8±24.6	31.8±25.6	0.2
Sperm normal morphology %	22.6±22.9	26.6±24.6	0.6

P value ≤ 0.05 is significant for T-test*.

underwent ICSI procedure yielded 18 positive pregnancies out of 21 participants. In comparison, participants with Oligospermia had four positive pregnancies out of 7 participants. Participants with Asthenospermia who underwent ICSI had only two positive pregnancies out of seven participants. Finally, patients with Teratozoospermia who underwent an ICSI procedure had seven positive pregnancies out of 15 participants, as shown in Table 4.

T-test of independent variables was done to investigate the difference in sperm parameters among the following two groups; 1- participants with normal levels of ASA d" 60 U/ml and 2- participants with abnormal ASA levels e" 60 U/ml. There was no significant difference in Sperm concentration levels (P value = 0.6), Progressive sperm motility (P value = 0.2), and Sperm normal morphology (P value = 0.6) among the two groups as illustrated in Table 5.

A T-test of independent variables was done to investigate the difference in ICSI outcome among the following two groups; 1- participants with normal levels of ASA dH 60 U/ml and 2-

Table 6. A comparison in ICSI outcome according to normal and abnormal distribution of ASA among the participants

ICSI outcome	Anti-Sperm Antibodies (ASA) ≤ 60 U/ml 34 participants (68%)	≥ 60 U/ml 16 participants (32%)	P value
Retrieved oocytes	11 ± 7	8 ± 5	0.2
MII oocytes	8 ± 6	7 ± 4	0.6
2PN	4 ± 3	4 ± 3	0.7
Fertilization rate	76 ± 19	84 ± 19	0.1
Cleavage rate	87 ± 20	87 ± 26	0.9

P value ≤ 0.05 is significant for T-test*.

Table 7. A comparison in sperm parameters according to pregnancy outcome (Positive pregnancy, & Negative pregnancy)

Sperm Parameters	Pregnancy Test Positive	Negative	P Value
Sperm concentration million/ml	48.5±28	41.9±28.2	0.4
Progressive sperm motility %	45.2±23.3	30±24.9	0.03*
Sperm normal morphology %	25±23	22.6±22.5	0.7

P value ≤ 0.05 is significant for T-test*.
No significant difference in ASA levels among the two groups (pregnancy positive, & pregnancy negative) (P value = 0.6) as shown in Table 8 The mean levels of ASA in pregnancy-positive patients were 68 ± 112 U/ml, while the mean levels of ASA in pregnancy-negative patients were 83 ± 111 U/ml.

Table 8. A comparison in Anti-Sperm Antibody levels according to pregnancy outcome (Positive pregnancy, & Negative pregnancy)

Variable	Pregnancy Test Positive (26)	Negative(24)	P Value
ASA	68 ± 112	83 ± 111	0.6

P value ≤ 0.05 is significant for T-test*.

Table 9. A comparison of ICSI outcomes among the two groups (Positive pregnancy, & Negative pregnancy)

ICSI outcome	Pregnancy Test Positive (26)	Negative(24)	P Value
Retrieved oocytes	10±6	10±7	0.9
MII oocytes	8±5	8±5	0.8
2PN	4±2	4±3	0.4
Fertilization rate	80.9±18	75.9±20.9	0.4
Cleavage rate	91.5±11	82±29	0.1

P value ≤ 0.05 is significant for T-test*.

participants with abnormal ASA levels eH 60 U/ml. There was no significant difference in the retrieved oocytes (P value = 0.2), mature oocytes MII (P value = 0.6), 2PN (P value = 0.7), fertilization rate (P value =0.1), and the cleavage rate (P value = 0.9) among the two groups as illustrated in Table 6.

A T-test of independent variables was conducted to investigate the difference in sperm parameters among two independent groups; 1- pregnancy positive, 2- pregnancy negative as shown in Table 7. It was found that there is no significant difference in sperm concentration (P value = 0.4), and sperm normal morphology (P value =0.7) among the two groups (Pregnancy positive and pregnancy negative).

However, the T test has shown a significant difference in progressive sperm motility (P value = 0.03) among the two groups as shown in Table 7.

T-test of independent variables was conducted to investigate the difference in ICSI outcome among the following two groups; 1- Positive pregnancy and 2- Negative pregnancy. It was found that there is no significant difference in the Retrieved oocytes (P value = 0.9), MII oocytes (P value = 0.8), 2PN (P value = 0.4), *Fertilization* rate (P value = 0.4), and the Cleavage rate (P value = 0.1) among the two groups of comparison as shown in Table 9.

DISCUSSION

Intra-cytoplasmic sperm injection (ICSI) was introduced in the early nineties (1992) and has drastically changed the treatment of advanced cases of male infertility [14]. The performance of ICSI procedures has expanded and revolutionized worldwide to treat infertile couples and not merely treat male infertility, which was initially developed for it[14]. Therefore, the development of the procedure makes it necessary to investigate the efficacy, safety, and factors that might influence ICSI outcomes, such as ASA, which is the main concern in the current study.

ICSI procedure was developed to overcome such issues. Therefore, the present study has analyzed the effects of ASA on ICSI outcomes.

In the current study, ICSI outcomes were compared according to the results of SFA, which were divided into four main groups (Normospermia, Oligospermia, Asthenospermia, & Teratospermai). It is worth mentioning that there was a significant difference in sperm features among the four groups: 1- sperm concentration (P value = 0.006) 2- progressive sperm motility (P value <0.001) 3- normal sperm morphology (P value <0.001).

A study was conducted to examine the ASA incidence and to evaluate its influence on sperm motility among 144 patients who have normo-zoospermic and abnormal semen [15]. The study has followed WHO guidelines and Kruger's strict criteria in comparable semen sample classification methods to the present study. The study has concluded that there is no association between the presence of ASA in semen samples and its quality, which agrees with the present study results as it was found that ASA is not correlated to sperm concentration (P value = 0.3), progressive sperm motility (P value = 0.1), and normal sperm morphology (P value = 0.3).

Moreover, the present study has shown that there was no relationship between semen ASA and any of the ICSI outcome parameters such as the number of retrieved oocytes, mature oocytes

(P value = 0.8, r = - 0.036), 2PN (P value = 0.9, r = - 0.003), fertilization rates (P value = 0.09, r = 0.238), and the cleavage rates (P value = 0.7, r = 0.062). The current study included a comparison in semen ASA levels between the two groups of successful ICSI outcomes (Pregnancy positive) and unsuccessful ICSI outcomes (Pregnancy negative); the comparison has shown that there is no difference in the semen ASA levels among both the groups (P value = 0.6) which agrees with the result of the meta-analysis and the present study finding (there is no association between semen ASA and pregnancy outcome among ICSI procedure). The results collected from the meta-analysis and the present study suggest that ICSI is considered viable treatment options for infertile couples where male partners have semen ASAs. On the contrary, similar findings cannot be generalized for infertile couples with semen ASAs who undergo IUI or try to conceive naturally [16].

A case-control study assessed the benefits of ASA screening by comparing ICSI outcomes [17]. The study included 100 couples screened for ASA by direct immune-bead assay for IgG, IgA, & IgM, almost similar to the present study screening tool (ASA ELISA immunoassay IgG, IgA, & IgM). The case-control study opted to divide the participant into three groups according to the WHO criteria (positive ASA e" 50%, intermediate ASA < 50%, & negative (control) ASA) and recorded the location of ASA titers on the sperm whether the head, mid-piece or the tail which this was not done in the present study.

The case-control study has confirmed no difference in most ICSI outcomes, such as the embryo cleavage and the clinical pregnancies among the three groups (positive, intermediate, & control), similar to the present study results. However, a negative correlation was observed between ASA titer and the fertilization rate in the case-control study among the three groups (the e" 50% positive had 68.1% fertilization. In contrast, the intermediate < 50% group had 83%, and the control 76.2%) 17. which contradicts our study finding as there was no relationship between ASA and the *fertilization* rates (P value = 0.09, r = 0.238). Moreover, the present study compared the sperm parameters and ICSI outcome according to the normal distribution of ASA (Cut-off point was 60 (34 participants d" 60 U/ml, & 16 participantse" 60 U/ml), and it was found that there was no difference in the sperm parameters and ICSI outcomes among the two groups. Even though the case-control study has shown a mild effect of ASA on the *fertilization* rate, the ASA had no effect on pregnancy outcome, which agrees with the current study findings, and this leads to the conclusion that ASA screening among infertile couples who are potential candidates for ICSI procedure has only marginal benefits [17].

Some studies supported our results regarding the negative effect of ASA on sperm motility, simply due to slowing down the sperm by joining of antibodies to the sperm tail [18,19]. other investigators did not find any correlation between the existence of ASA and sperm motility [20]. while a third section of researchers found this negative effect of ASAs on motility to be related to the severity of autoimmunization [21]. In the present study despite there was difference in motility between positive and negative ASA patients, but this difference was not significant.

A retrospective study was conducted at the Egyptian IVF-ET Centre to investigate the relationship between semen parameters and the fertilization and pregnancy rates of 130 ICSI cycles done to manage male factor infertility [22]. The total number of achieved clinical pregnancies in the study was 46 (35%), while in the present study, the total number of achieved clinical pregnancies was 26 (52%). The retrospective study has concluded that in ICSI, the fertilization and pregnancy rates are not influenced by various semen parameters presuming the sperms with normal morphology (well-shaped) were used for the procedure, and this result is almost comparable with the present study findings. The pregnancy rates among the four included groups in the present study were as follows: 1- Normospermia (61%) 2-Oligospermia (57%) 3-Asthenospermia (28%) 4-teratospermia (46%). Moreover, this study has shown that progressive sperm motility affects pregnancy outcomes. There was a significant difference (P value = 0.03) between patients with positive pregnancy and negative pregnancy outcomes, which might explain the lowest pregnancy rates among Asthenozoospermic patients (28%).

Major limitation of the current study is the relatively small number of ASA-positive patients, we strongly encourage further studies with larger

groups of patients, in order to confirm that ICSI outcomes are not impaired by ASA

CONCLUSIONS

Intra-cytoplasmic sperm injection ICSI is an effective and well-developed method for managing male factor infertility. pregnancy rates were higher in the normospermia group than the other three groups. An insignificant effect was seen between ASA and sperm parameters. And lastly, no relationship between ASA and ICSI outcomes. Because ICSI overcomed the presence of ASA in the semen plasma

ACKNOWLEDGEMENT

I would like to express our gratitude to the staff of the fertility centre in Najaf province for their efforts and assistance.

Conflict of Interest

I/We certify that I/We have no Conflict of interest.

Funding source

There are no funding Sources

REFERENCES

1. Taebi M, Kariman N, Montazeri A, Alavi Majd H, Jahangirifar M. Development and psychometric properties of Female Infertility Stigma Instrument (ISI-F): A sequential mixed method study. *BMC Womens Health*. 2022;22(1):557. Published 2022 Dec 30
2. Taebi M, Kariman N, Montazeri A, Alavi Majd H. Infertility Stigma: A Qualitative Study on Feelings and Experiences of Infertile Women. *Int J Fertil Steril*. 2021;15(3):189-196.
3. Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*. 2010;16(3):231-245.
4. Agarwal, A., S. Baskaran, N. Parekh, C.-L. Cho, R. Henkel, S. Vij, M. Arafa, M. K. P. Selvam and R. Shah (2021). "Male infertility." The Lancet 397(10271): 319-333.
5. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. *J Hum Reprod Sci*. 2015;8(4):191-196.
6. World Health Organization, WHO laboratory manual for the examination and processing of human semen, 5th ed. World Health Organization. (ý2010).retrived from https://apps.who.int/iris/handle/10665/44261
7. Daneshmandpour Y, Bahmanpour Z, Hamzeiy H, et al. MicroRNAs association with azoospermia, oligospermia, asthenozoospermia, and teratozoospermia: a systematic review. *J Assist Reprod Genet*. 2020;37(4):763-775.
8. Maroto-Morales A, García-Álvarez O, Ramón M, et al. Current status and potential of morphometric sperm analysis. *Asian J Androl*. 2016;18(6):863-870.
9. Kherraf ZE, Cazin C, Lestrade F, et al. From azoospermia to macrozoospermia, a phenotypic continuum due to mutations in the *ZMYND15* gene. *Asian J Androl*. 2022;24(3):243-247.
10. Cui D, Han G, Shang Y, et al. Antisperm antibodies in infertile men and their effect on semen parameters: a systematic review and meta-analysis. *Clin Chim Acta*. 2015; 444:29-36.
11. Rubino P, Viganò P, Luddi A, Piomboni P. The ICSI procedure from past to future: a systematic review of the more controversial aspects. *Hum Reprod Update*. 2016;22(2):194-227.
12. Devroey P, Van Steirteghem A. A review of ten years' experience of ICSI. *Hum Reprod Update*. 2004;10(1):19-28.
13. Baczkowski T, Kurzawa R, G³abowski W. Methods of embryo scoring in in vitro fertilization. *Reprod Biol*. 2004;4(1):5-22.
14. Tarlatzis BC, Bili H. Survey on intracytoplasmic sperm injection: report from the ESHRE ICSI Task Force. *Human Reproduction*. 1998;13(suppl_1):165-177.
15. Munuce MJ, Berta CL, Pauluzzi F, Caille AM. Relationship between anti-sperm antibodies, sperm movement, and semen quality. *Urol Int*. 2000;65(4):200-203.
16. Zini A, Fahmy N, Belzile E, Ciampi A, Al-Hathal N, Kotb A. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. Human Reproduction. 2011;26(6):1288-1295.
17. Baek K, Neri Q, Wang A, Bongiovanni A, Rosenwaks Z, Palermo G. Has the Advent of ICSI Made Antisperm Antibody Testing Obsolete? *Fertility and Sterility - FERT STERIL*. 09/01 2005;84.
18. Verón GL, Molina RI, Tissera AD, Estofan GM, Marín-Briggiler CI, Vazquez-Levin MH. Incidence of Sperm Surface Autoantibodies and Relationship with Routine Semen Parameters and Sperm Kinematics. *Am J Reprod Immunol*. 2016;76(1):59-69.
19. Rossato M, Galeazzi C, Ferigo M, Foresta C. Antisperm antibodies modify plasma membrane

functional integrity and inhibit osmosensitive calcium influx in human sperm. *Hum Reprod*. 2004;19(8):1816-1820.

20. Garcia PC, Rubio EM, Pereira OCM. Antisperm antibodies in infertile men and their correlation with seminal parameters. *Reprod Med Biol*. 2007;6(1):33-38. Published 2007 Feb 16.
21. Barbonetti A, Castellini C, D'Andrea S, et al. Prevalence of anti-sperm antibodies and relationship of degree of sperm auto-immunization to semen parameters and post-coital test outcome: a retrospective analysis of over 10 000 men. *Hum Reprod* 20193; 4: 834–841.
22. Mansour RT, Aboulghar MA, Serour GI, Amin YM, Ramzi AM. The effect of sperm parameters on the outcome of intracytoplasmic sperm injection. *Fertil Steril*. 1995;64(5):982-986.