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A Comparison of ICSI Outcomes and Reactive Oxygen Species Levels in Seminal Plasma between Normozoospermia and Sperm Abnormalities Groups for Infertile Men

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Abstract

Background: Social life and the healthcare systems are affected by infertility. Infertility could be primary or secondary; secondary infertility is the inability to conceive after one successful pregnancy while primary infertility is the inability to conceive at all. As sperm abnormalities cause male infertility, Intracytoplasmic Sperm Injection (ICSI) is a particular type of in vitro fertilization (IVF) used to treat severe male-factor infertility. Free radical oxygen derivatives are reactive oxygen species (ROS). Reactive oxygen species may cause 30% to 80% of male infertility. **Aims of the study:** the present study aims to determine the reactive oxygen species level in seminal plasma and to study the Intracytoplasmic Sperm Injection outcomes for Normospermia group and compare it with Oligozoospermia, Asthenozoospermia and the Teratozoospermia Groups. **Material, and Methods:** a cross-sectional study was conducted between January 2023 to June 2023 in Najaf, on a non-random sampling including 50 couples who suffered from a minimum of 12 months of primary fertility with regular unprotected sexual intercourse and who had attended the fertility center in Najaf requesting fertility treatment. A gynecological examination and assessment were done for the female participants while the urologists examined and assessed the male subjects; then, a semen analysis was carried out followed by macroscopic and microscopic examinations. After the preparation of the sample for ICSI and performing ICSI, pregnancy was assessed and the outcomes of the different groups were compared. At the same time, reactive oxygen species levels were assessed by using the Human Reactive Oxygen Species ELISA kit. Then, the SPSS version 26 was used to perform the statistical analysis. **Results:** Among the twenty-one patients with Normozoospermia, seven with Asthenospermic, seven with Oligozoospermia and fifteen with Teratozoospermia, there was a significant difference among the four groups regarding sperm concentration, progressive motility, and sperm normal morphology. There was no significant difference among the type of sperm, Normo, Astheno, Tearato, and Oligozoospermia, regarding birth rate and ICSI outcomes. Males with higher progressive motility showed a higher pregnancy rate. **In conclusion**, despite the presence of reactive oxygen species, Intracytoplasmic Sperm Injection was advantageous and effective for all four groups. reactive oxygen species had no significant effect on Intracytoplasmic Sperm Injection outcomes, however Normozoospermia was associated with greater pregnancy rates. Despite the presence of reactive oxygen species, Intracytoplasmic Sperm Injection was advantageous and effective for all four groups. ROS had no significant effect on Intracytoplasmic Sperm Injection outcomes, however Normozoospermia was associated with greater pregnancy rates.

Keywords: Infertility, Intracytoplasmic Sperm Injection, Najaf, Reactive Oxygen Species.

INTRODUCTION

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]. Infertility is either primary or secondary [3]. Primary infertility is when a pregnancy has never been achieved by an individual, while secondary infertility is the inability to conceive after at least one prior successful conception [4]. Infertility in men is typically brought on by issues with the ejection of semen, a lack of sperm or low sperm counts, or poor sperm shape (morphology) and motility [5]. Environmental, occupational, and lifestyle habits can degrade semen quality and cause male infertility [6].

Semen analysis continues to be the cornerstone investigation for male infertility, regardless of its weakness [7]. It is performed with high standards to evaluate the ejaculate's descriptive parameters [8]. Normospermia is characterized by having normal seminal fluid analysis values across all ejaculate parameters according to WHO's 2021 semen normalcy guidelines. Men with normal sperm parameters, often known as normospermia, may have infertility due to idiopathic, endocrine, or hormonal reasons, congenital or acquired urogenital abnormalities, or extramarital sexual dysfunction [9].

Sperm abnormalities are an essential factor in male infertility. Low sperm count is known as "oligozoospermia" (15 million sperm/mL) [10]. Teratozoospermia refers to an abnormal morphology of spermatozoa in more than 85% of the sperm. The term "monomorphic teratozoospermia" is used when all the spermatozoa exhibit a distinct abnormality [11].

Asthenozoospermia is a term referring to poor sperm motility or an absence of sperm motility in the analysis of semen samples. It is considered a major factor in men's infertility [12]. Abnormalities related to sperm motility are another type of sperm issue that causes infertility. Rapid progressive motility of a minimum of 25 m/s plays a significant role in the effective passage of spermatozoa through the cervical mucus.

Reactive oxygen species (ROS) are oxygen derivatives and free radical-containing molecules. Free radicals are molecules that are not paired with electrons and are extremely reactive, attempting to reach an electronically stable form. It has been reported by Wagner et al. that reactive oxygen species might be a causative factor in 30% to 80% of infertility cases in men's cytoplasm [13].

Many studies suggested oxidative stress, a condition characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense systems, as a new emerging factor in unexplained male infertility [14,15,16,17].

Spermatozoa and polymorph-nuclear leukocytes produce the most reactive oxygen species in semen. DNA fragmentation in spermatozoa is linked to low-quality semen. The fraction of spermatozoa with fragmented DNA negatively affects IVF and ICSI fertilization rates. Infertile men fragment sperm DNA for unknown reasons. However, reactive oxygen species (ROS) are known to cause somatic cell DNA damage, therefore they may be linked to germ cell DNA fragmentation [13].

Intracytoplasmic Sperm Injection (ICSI) is a specially designed form of in vitro fertilization (IVF) used mainly to manage severe cases of male-factor infertility; it involves the injection

of a single sperm that has been surgically retrieved from the epididymis or testis into a mature egg (10). According to WHO 2021 standards for normal sperm, Normospermia is defined by having normal seminal fluid analysis values for all ejaculate parameters. Men with normal sperm parameters, known as normospermia, might suffer from infertility that can be either idiopathic, endocrine, hormonal, congenital or acquired urogenital abnormalities or sexual dysfunction extra [(9)].

This study aimed first to determine the reactive oxygen species level in seminal plasma and to study the ICSI outcomes for the Normospermia group and compare it with Oligozoospermia, Asthenozoospermia, and the Teratozoospermia Groups. Secondly, it aims to study the relationship between ROS, sperm parameters and the ICSI outcomes.

Material and Methods

Study design and sample collection

This cross-sectional study was performed at the Fertility Centre/ Al Sadr Medical City / Najaf for six months, from Jan 2023-June 2023. Patients with Normospermia, Oligozoospermia, Asthenozoospermia, and Teratozoospermia, in line with WHO standards 2021, were included in the study. The study 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. Gynecological examination and assessment were done for the female participants while the male subjects were examined and assessed by the urologists.

The inclusion criteria for male participants include Normospermia, Oligospermia, Asthenospermia and Teratozoospermia *while inclusion criteria for the female partners of the participants*: healthy female partners, Young female partners 21<age< 43 years old).

The exclusion criteria for male participants include: severe Oligozoospermia, Severe Asthenozoospermia , Severe Teratozoospermia while the exclusion Criteria for the Female Partners of the Participants: > 43 years old, & <21 years old), Overweight (BMI ≥ 30 kg/m²), Underweight female partners (BMI < 18.5 kg/m²), Female partners with polycystic ovarian syndrome (PCOS) and Female partners with Ovarian hyper-stimulation syndrome (OHSS).

Sperm classification diagnosis for males was reached by performing two seminal fluid analysis tests of two to three months' intervals depending on the (WHO criteria 2021). The participant's hormonal profiles (FSH, LH, testosterone, & Prolactin) were retrieved from the case records. No exclusion criteria were implemented regarding previous fertility treatment outcomes. Then ROS levels, ICSI outcomes ((Retrieved oocytes, MII oocytes, 2PN, Fertilisation rate, Cleavage rate and Pregnancy Ratio) for Normospermia and Oligozoospermia, Asthenozoospermia and Teratozoospermia patients.

Semen sample processes and Techniques:

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.

Then macroscopic examinations of the seminal fluid, including liquefaction, viscosity, appearance, volume, and PH, were performed, followed by microscopic examinations of

concentration, motility, and morphology of the sperm. Then cells other than sperm that were present in the ejaculate were separated and counted, depending on WHO 2021 (Sunder & Leslie, 2021

Next, the sperm sample was prepared for ICSI by using the centrifugation swim-up technique for sperm preparation. These have been summarized by the following steps:

- The tube was centrifuged at 3000 rpm for 10 minutes,
- the supernatant was aspirated without disturbing the pellet, and then
- a resuspension took place in 0.3–0.5 mL of media.
- The conical tube was put in the incubator at a 45° angle for 30 minutes at 37°C to allow the sperm to swim up.
- Following the incubation period, an aspiration of the supernatant was done without disturbing the upper layer that accommodates the highest amount of motile sperm.
- Slide analysis was performed by taking a slight drop.
- After that, ROS were by assessed using an ELISA kit. Following manufacture instructions

Preparation for ICSI:

An inverted microscope, type Olympus Optical Co Ltd., Japan, which is provided by micro-injectors, a magnificent lens, with a capability of 200x and 400x, micromanipulators ("RI, UK"), and a three-dimensional maneuverer for both coarse and refined movements were employed for the process. The temperature was maintained at 37 °C by a heating surface on the inverted microscope.

Sperm immobilization:

Sperms that looked motile and had normal morphology were selected for immobilization. An aspiration of the selected sperm took place by injection pipette "Cook, Australia" to the PVP droplet for immobilization.

Oocyte Injection:

The oocyte was placed in a suction connected to the attached pipette (Cook, Australia). The immobilized spermatozoa were pushed inside the cytoplasm of the mature oocyte MII at 3 o'clock. Lastly, a small suction of the ooplasm was done after the introduction of the oocyte to guarantee that the injector pipette was inside the cytoplasm.

Incubation of injected oocytes:

The preparation of the culture dish took place 24 hours before using the oocyte injection. Then, the injected oocytes were moved to droplets through an automatic pipette and stored in a CO2 incubator until the next day to assess fertilization.

Fertilization Assessment:

This assessment took place on the morning of the next day before the injection of the mature oocytes. Injected oocytes that manifested two pronuclei and two polar bodies were deemed to have been fertilized successfully, while others were neglected.

Embryo selection and transfer:

Morphological evaluation of the embryos took place 24–48 hours after the oocytes had been retrieved. The morphological assessment included the existence of the first cleavage, the symmetry of blastomeres, and the magnitude of fragmentation. Good-quality embryos, grade I, selected for transfer have symmetrical blastomeres with no trivial cytoplasmic fragments. Grade II followed the good-quality embryos for transfer as they also have symmetrical blastomeres but with a minimum amount of cytoplasmic fragments. Finally, grade III embryos were transferred that have asymmetrical blastomeres and fragmentation, which is not greater than 50%. Other embryos that displayed higher grading should have been addressed. The maximum number of transferred embryos was three to four; the best-

selected embryos were transferred 48 to 72 hours after the retrieved oocyte.

Pregnancy examination:

Pregnancy was confirmed by an elevation in serum HCG hormone concentrations, known as a positive pregnancy test. The pregnancy test was done at least 12 days after the embryo selection and transfer.

Statistical analysis:

Version 26 of SPSS was used for the statistical analysis (Inc., Chicago, IL, USA); mean and standard deviation were used for parametric data; one-way ANOVA was used to compare between categorical independent and numerical dependent variables; and Chi-Square was used to compare between categorical variables. A P value of 0.05 was considered statistically significant.

Ethical approval

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. Additionally, verbal consent was obtained by all of the included patients or their parents prior to participation, as they gave their approval to participate in this study by giving their samples and providing the information that is needed.

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), Table no. 4.1.

Regarding sperm parameters variables among the four groups (Normo, Oligo, Astheno, Terato), a One-way ANOVA test was performed. It has shown that there is 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 no. 4.1.

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) Per cent 42%	Oligo (N=7) Per cent 14%	Astheno (N=7) Percent 14%	Terato (N=15) Per cent 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/year	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	7.5 ± 5.6	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**.					

There was no significant difference in ROS levels among the two groups (Astheno & Normo) (*P* value = 0.6. There was no significant difference in ROS levels among the two groups (Terato & Normo) (*P* value = 0.2). as shown in Table2

Table 2: ROS levels in the seminal fluid samples of oligospermia, Asthenospermia, & Teratospermia patients compared to the samples of Normospermia patients.

Variable	Sperm classification	Number	Mean	P value
ROS	Normospermia Oligospermia	21 7	400.4 505.5	0.7
ROS	Normospermia Asthenospermia	21 7	400.4 506.2	0.6
ROS	Normospermia Teratozoospermia	21 15	400.4 259.8	0.2

Pearson's rank correlation coefficient test was consulted to investigate the relationship between ROS and (sperm concentration, progressive sperm motility, Normal Sperm Morphology, number of oocytes, and mature oocytes) in the studied samples. It has been found that there is no relationship between ROS and five other parameters as follows: (P value = 0.5, r = 0.09), (P value = 0.9, r = 0.009), (P value = 0.9, r = 0.003), (P value = 0.09, r = 0.243) and (P value = 0.07, r = 0.261) regarding sperm concentration, progressive sperm motility, Normal Sperm Morphology, number of oocytes, and mature oocytes respectively, as shown in Table. 3.

Table 3: The relationship between ROS & sperm parameters and oocytes numbers.

First variable	Second Variable	R value	P value
ROS	Sperm Concentration	0.09	0.5
ROS	Progressive Sperm Motility	0.009	0.9
ROS	Normal Sperm Morphology	0.003	0.9
ROS	Number Of Oocytes	0.243	0.09
ROS	Mature Oocytes	0.261	0.07
P value ≤ 0.05 is significant for Pearson's rank correlation coefficient test*.			

Pearson's rank correlation coefficient test was consulted to investigate the relationship between ROS and (the fertilized oocyte (2pn), the fertilization rate, the total number of embryos, and the number of transferred embryos) in the studied samples (50 participants). It was found that there is no relationship between ROS & the four comparison groups) (P value = 0.4, r = 0.130), (P value = 0.2, r = - 0.183), (P value = 0.5, r = 0.096) and (P value = 0.4, r = 0.134) regarding the fertilized oocyte (2pn), the fertilization rate, the total number of embryos, and the number of transferred embryos respectively as shown in Table 4

Table 4: The relationship between ROS & (fertilized oocyte (2pn), fertilization rate, number of embryos).

First variable	Second Variable	r value	P value
ROS	Fertilised Oocyte (2pn)	0.130	0.4
ROS	Fertilisation Rate	- 0.183	0.2
ROS	Total Number of Embryos	0.096	0.5
ROS	The Number of Transferred Embryos	0.134	0.4
P value ≤ 0.05 is significant for Pearson's rank correlation coefficient test*.			

Pearson's rank correlation coefficient test was performed to investigate the relationship between ROS and ICSI outcomes, the number of good embryos, the cleavage rate, the pregnancy ratio, and the oocyte number, in the studied samples. It has been found that there is no relationship between ROS & ICSI outcomes as follows (P value = 0.07, r = 0.257), (P value = 0.7, r = 0.052), (P value = 0.4, r = 0.118) and (P value = 0.4, r = - 0.111) regarding the number of good embryos, the cleavage rate, the pregnancy ratio, and the oocyte number respectively, as shown in Table. 5

Table 5: The relationship between ROS & the number of good embryos and cleavage rate.

First variable	Second Variable	r value	P value
ROS	Number of Good Embryos	0.257	0.07
ROS	Cleavage Rate	0.052	0.7
ROS	Pregnancy Ratio	0.118	0.4
P value ≤ 0.05 is significant for Pearson's rank correlation coefficient test*.			

Table.6 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 was done, and 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, 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 was done to investigate the difference in the pregnancy ratio between the four groups, and it was found that there was no significant difference in the pregnancy ratio (*P* value =0.5). Participants with Normospermia samples who 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,

Table. 6.: ICSI outcome 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
MII oocytes	8.9±5.6	5.9±2.5	8.3±6.8	7.8±5	0.6
2PN	4.8±3.1	3.4±1.6	3.7±2.5	3.8±2.7	0.6
Fertilisation rate	72.9±19	88.6±18.6	86±13	78±21	0.2
Cleavage rate	84.4±22.7	94±10.3	85.7±37.8	88±16.5	0.8
Pregnancy Ratio	Pregnancy:61.9%	pregnancy:57.1%	Pregnancy:28.6%	Pregnancy: 46.7%	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**.

Discussion

ICSI was introduced in the early nineties (1992) and has drastically changed the treatment of advanced cases of male infertility. 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 [19]. Therefore, the development of the

procedure makes it necessary to investigate the efficacy, safety, and factors that might influence ICSI outcomes, such as ROS, which are the main concern in the current study.

Couples included in the study have similar sociodemographic variables, such as weight, age, partners' age, and years of infertility, and similar ovarian reserve features, the total number of retrieved oocytes, number of transferred oocytes, mature MII oocytes, and the various grades embryos were similar among the participants, and that was done to limit the effects of any confounding factors that might affect ICSI outcome and concentrate mainly on male factors of infertility.

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).

The study aims to investigate the relationship between ROS and sperm features, the results of the present study have shown that there are ROS levels in the semen of the participants did not affect the sperm parameters (sperm concentration (P values = 0.5), progressive sperm motility (P values = 0.9), and normal sperm morphology (P values = 0.9)) and it is almost the same among the four included groups (Normospermia, Oligospermia, Asthenospermia, Teratospermia).

However, the results from the present study contradict a prospective clinical study that investigated the relationship between (ROS) production and sperm morphology among infertile men [20]. The prospective clinical study has concluded that sperm ROS production was inversely related to the proportion of normal sperm morphology and borderline morphology. Additionally, ROS production was related directly to the proportion of sperm amorphous heads, faulty acrosomes, and mid-piece defects.

The prospective study has concluded that high ROS concentration is associated significantly with reduced sperm concentration and the percentage of motile sperm in semen. This result disagrees with the present study results that show no association between ROS and sperm features. The present study included only 8 participants (16%) with high ROS levels (≥ 400) and 42 participants (84%) with low ROS levels (≤ 400), while in the prospective study, 84% of the included individuals had high ROS levels, and 85% of included individuals had low ROS levels. These differences might explain the contradiction in the results of the two studies due to the lower sample size recruited in the current study.

According to a study conducted by (Agarwal), reactive oxygen species are inversely connected with classical sperm characteristics such as concentration, motility, and morphology. This is not the same result as the current study, which found no significant association between ROS and sperm parameters (see Table3). This disparity could be attributed to (Agarwal's) usage of a lower ROS cut-off level of 91.9 RLU/s.

A study was performed by [21] to compare the concentration of reactive oxygen species (ROS) and total antioxidant (TAS) in seminal plasma among two groups of IVF and ICSI patients to determine these effects on sperm quality and IVF/ICSI outcome. The study included 48 participants (26 underwent IVF, & 22 underwent ICSI), while the present study included 50 couples who underwent ICSI procedures only. The sperm parameters were evaluated one-hour post ejaculation (sperm morphology, maturity, & DNA strand breaks). Some of the interesting findings, there was a negative correlation between seminal plasma ROS concentration and sperm vitality (r =

−0.111, P = 0.453) and morphology (−0.141, P = 0.340) [13]. However, in this study, we measured sperm concentration in a million/ml, progressive sperm motility%, & sperm morphology % for sperm parameters. It was found that there is no relationship between ROS and sperm parameters (P value = 0.5), (P value = 0.9), (P value = 0.9), respectively.

The present study compared sperm parameters according to normal ROS distribution (400 was the cut-off point, 42 participants ≤ 400, & 8 Participants ≥ 400), and there was no difference between the two groups in all measured sperm parameters sperm concentration million/ml (P value = 0.3), progressive sperm motility % (P value = 0.1), & sperm normal morphology % (P value = 0.4). The contradiction in this result can be explained by the fact that the collected semen was washed before the ICSI procedure, which diminishes ROS concentration from the sperm. In contrast, in the mentioned study, ROS concentration might refer to the unwashed seminal plasma (before washing the samples). Furthermore, in the mentioned study, the fertilization rates were comparable in both groups (IVF & ICSI) (67. 26 vs 67.26), while the relationship between ROS concentration and fertilization rate was n. In contrast, (r = − 0.029, P = 0.045) contradicts our findings as we found no correlation between ROS and the fertilization rate (P value = 0.2, r = - 0.183). Despite the differences in the methodologies and some findings from the two studies, it was concluded in the mentioned study that ROS concentration hurts spermatozoa quality but doesn't affect the fertilization rate in IVF/ICSI, which is a comparable result to the present study.

This may have been due to the small sample size; a study with a larger sample size and longer duration could have produced meaningful results.

There were some limitations that countered the current study and resulted in a lower sample size: first, since we were collecting semen samples from patients who were attending the fertility center/Al-Sader Medical City sometimes the center was shut down due to financial issues, thus lower sample size was collected. the second limitation is the short duration of the study (6-9 months only).

Conclusion

The current study concluded that: The Normozoospermia patients group had a higher pregnancy rate than the other three groups. The ICSI technique is a successful way to overcome the ROS presented in the semen plasma. There was a negative correlation between ROS and (the motility of Sperms, Fertilization rate) .

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