International Journal of Clinical Obstetrics and Gynaecology

ISSN (P): 2522-6614
ISSN (E): 2522-6622

www.gynaecologyjournal.com
2025; 9(1): 156-163
Received: 12-11-2024
Accepted: 19-12-2024

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Association of serum and follicular fluid thiol/disulfide homeostasis in patients undergoing intracytoplasmic sperm injection

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DOI: https://doi.org/10.33545/gynae.2025.v9.i1c.1578

Abstract
Background: Despite the fact that physiological amounts of reactive oxygen species (ROS) are essential for oocyte maturation, physiological follicular atresia, ovulation, and fertilization, excessive ROS affects normal reproductive function. Thiols are antioxidants that contain sulfhydryl groups and act as electron acceptors for ROS. Thiols neutralize oxidants, converting them into less harmful compounds and forming disulfide molecules. Thiol/disulfide pools play an important role in intracellular redox equilibrium, which is required for antioxidant defense.
Objective: Assess serum and follicular fluid thiol/disulfide homeostasis in patients with normal, hyper-, and poor responses to intracytoplasmic sperm injection.
Study design: A retrospective cohort study.
Setting: Fertility Center of Al-Sadder Teaching Hospital Al_Najaf Governorate & High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrein University, Al Kadumiayh, Baghdad, Iraq. The study run from the first of January 2024 until the end of October 2024.
Patients and Methods: Following a complete history, examination, investigation, and ovarian response, 50 infertile patients with a history of primary and secondary infertility were divided into three groups: normal responders, hyper responders, and poor responders. Serum and follicular fluid was collected on the day of oocyte pick-up, and measurements were done for the total thiol, native thiol, and disulfide levels. The quality of the oocytes and embryos as well as the results of the intracytoplasmic sperm injection were monitored for each patient.
Results: There is no significant association between serum and follicular fluid thiol/disulfide homeostasis in normal, hyper and poor responders.
Conclusion: In this study, none significant difference was found in the antioxidant thiol/disulfide homeostasis in normal responders, hyperresponders, and poor responders. Therefore, further studies are needed for larger sample sizes, using different criteria and protocols.

Keywords: Normal responder, hyperresponder, poor responder, ovarian reserve, thiol/disufide homeostasis, antioxidant, oxidant stress

Introduction
The inability to achieve a clinical pregnancy following a year or more of regular, unprotected sexual activity is known as infertility [1]. ART patients can feel secure with the knowledge that their chances of a live birth are high. The live birth rate was 80% for women under 35, 61% for those between 35 and 39, and 26% for those over 40. This emphasizes how important it is to provide ART patients an age-specific prognosis when they are receiving counseling [2] Intracytoplasmic sperm injection (ICSI) is an IVF variant that works well for male factor infertility. A single sperm is directly inserted through the zona pellucida and egg cell membrane after the cumulus cells surrounding an ovum are enzymatically broken down during the ICSI micromanipulation procedure. ICSI has enabled azoospermic males to conceive with their partners. Sperm are mechanically removed from the testicle or epididymis in these situations [3]. Nowadays, a number of cofactors, including the function of micronutrients, influence the success of an ICSI cycle [4].

Individualized patient for ovarian stimulation
Controlled ovarian stimulation (COS), which aims to produce multi-follicular development to

have a good probability of transferring embryos with the highest implantation potential, continues to be the corner stone of successful ART treatment despite the exponential surge in technical advancements [5]. In order to determine the best individualized controlled ovarian stimulation (iCOS) strategy and ensure the shortest time to pregnancy and live birth, as well as a low risk of developing ovarian hyper stimulation syndrome (OHSS), most clinicians use ovarian reserve markers such as antral follicle count (AFC) and/or Anti-Müllerian hormone (AMH) for clinical decision-making [6].

In 2011, an ESHRE consensus group [7] established the so-called ESHRE Bologna criteria in an attempt to standardize the definition of Poor Ovarian Resonder. The Bologna POR population's variability was much reduced by the new POSEIDON (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) classification of the "low prognosis patient" [8, 9], which offers a more thorough stratification of anticipated low responders. Based on quantitative and qualitative factors, patients are classified into four subgroups according to: (i) age; (ii) antral follicle count and/or AMH; and (iii) ovarian response, if a prior stimulation was carried out [10, 11]. The hyper responder group (age <30 years, polycystic ovaries, lean body habitus, previous cycle with a high response are the high responders to gonadotrophin stimulation because they will generate a significant number of follicles that predispose to OHSS. Both AMH and AFC correlate strongly with one another and have a good prediction value for high responders [12].

Oxidative Stress in Female Infertility and Assisted Reproduction Technique

Oxidative stress is thought to have a negative physiological and pathological impact on a variety of reproductive processes, including folliculogenesis, oocyte maturation, sperm Deoxyribonucleic acid (DNA) damage, necrozoospermia, asthenospermia, endometriosis, and the cause of defective embryo development. Frequently affects the cellular membrane, retards embryo development, and causes cellular death, resulting in fragmented embryos with limited potential to implant. An correlation between OS and infertility is suspected, but a true cause-and-effect relationship has yet to be determined [13]. Reactive oxygen species can originate directly from gametes and embryos (Endogenous sources) or from their environment (Exogenous sources). Several external variables found in culture medium promote ROS formation in embryos, including oxygen content, the presence of metallic cations, and visible light exposure. Spermatozoa may also contribute to the creation of ROS during assisted reproduction techniques. Elevated ROS levels influence gametes, gamete interaction, fertilization, and pregnancy rates in IVF/ICSI. Oxidative injury to embryos can cause 2-cell block, embryonic arrest, or even embryonic death. Higher levels of ROS in the follicular fluid and semen are related with poor reproductive results with assisted reproduction. ROS in semen has been observed to dramatically affect the fertilization rate with IVF. ROS levels can be measured to help patients understand the negative consequences [13].

Thiola_Disulifde Haemostasis

While some quantities of oxygen radicals are necessary for optimal reproduction, higher levels of oxygen radicals harm reproductive cells [14] including proteins, lipids, nucleic acids, and carbohydrate structures. This causes harm to mitochondrial activity, reducing energy generation which has a negative impact on oocyte maturation, and oocyte fertilization [15].

Thiols belong to a class of chemical substances called mercaptans, and every OS condition in cells depends critically on their forms that include sulfhydryl groups [16]. They create disulfide bonds after entering the oxidative process via oxidants [17]. The thiol/disulfide ratio is crucial for antioxidant protection, detoxification, signal transduction, enzymatic activity modulation, apoptosis, and cellular signaling pathways [18]. Granulose cells produce the follicular fluid (FF) [19], which is made up of proteins, polysaccharides, steroid hormones, metabolic products, ROS, and antioxidants that influence folliculo-genesis. To maintain the FF's ROS levels at a suitable level, enzymatic antioxidant mechanisms are required. During follicle development, the follicular fluid is thought to serve as a communication medium between oocytes and follicular cells; variations in the follicular fluid content may alter the physiology of oocyte maturation and embryonic development, or even result in an anomaly in this physiological process [20]. After ROS-induced aging, oxidative cellular damage rises [21].

TOLA *et al*. (2018) studied the function of follicular fluid thiol/disulfide homeostasis in PCO is discussed, and it is concluded that worsening of thiol/disulfide homeostasis, particularly higher disulfide levels, may be one of the etiopathogenic pathways in polycystic ovarian syndrome. In their study, disulfide levels, as well as the disulfide/native thiol and disulfide/total thiol ratios that reflect the OS, were found to be higher in the PCOS group than in the non-PCOS group, whereas native thiol levels and the native thiol/total thiol ratio, which are antioxidant system markers, were found to be lower in the PCOS group. These data led us to believe that the thiol/disulphide ratio has changed toward OS in the PCOS group [22].

Another study conducted in 2020 by Ayse Z. Ozdemir *et al*. examined whether thiol/disulfide homeostasis had an impact on the quantity of metaphase 2 oocytes during in vitro fertilization treatment. They conclude that the isolated disulfide level and M2 number have a positive connection, indicating low-impact oxidative stress and a noninvasive way to detect it [23].

In 2023, Kadriyr Erdogan *et al*. found that FF and serum native and total thiol levels, antioxidant system markers, were significantly lower in the diminished ovarian reserve (DOR) group than in the control group [24].

The Esengul Turkylmize 2024 Study compared the thiol/disulfide balance, myeloperoxidase, and ischemia-modified albumin levels in the follicular fluid (FF) of poor ovarian response (POR) and normal ovarian response (NOR) women who received intracytoplasmic sperm injection (ICSI), concluding that disulfide levels differed significantly between the NOR and POR groups. The NOR group had significantly fewer metaphase II oocytes and a lower percentage of good-quality embryos than the POR group, which could be attributed to higher disulfide levels. Total thiol levels associated with the total of 2PN oocytes [25].

Aim of the Study: Investigating serum and FF thiol/disulfide homeostasis in patients receiving intracytoplasmic sperm injection (ICSI) treatment who were normal, poor responders, and hyper-responders was the aim of this study.

Patients and methods

This prospective cohort study was conducted in both Fertility Center at Al-Ssader General Teaching Hospital in Al_Najif Alshraff and the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al Nahrein University in Al Kadumiayh, Baghdad, Iraq. The study was carried out over a

ten-months period, beginning on January 1, 2024 and ending October 31, 2024. The Ethics Committee of the Scientific Council of the Arab Board of Iraq accepted the study protocol. All participants provided verbal consent.

Study population: A total of 50 infertile women were assessed and distributed into three groups: age, AMH, and ovarian response (posidon for poorresponder). The first group (22 normal responders) had a mean age of (30.18±1.8), AMH levels above 1.2 ng/ml, and excellent ovarian response (>9-16 oocytes). The second group included 19 infertile women who were diagnosed as poor responders, with a mean age (36.2±1.)7, AMH levels <1.2ng/ml, ovarian response ≤ 4 oocytes, or suboptimal 4-9 oocyte. The third group (9) of infertile women diagnosed as hyperresponders with mean age (26.1±1.1), AMH>3.5 ng/ml, and ovarian response ≥ 16 oocytes.

Patients' collection: All of the patients had a history of primary or secondary infertility, they were gathered from infertility centers. We employed a questionnaire format to assess several of the dependent variables, including patient age, main or secondary infertility duration, cause of infertility prior therapy, and IVF trials. The patients were selected based on straightforward selection criteria. Following the examination, the second day of the cycle's hormonal profile—which included FSH, LH, AMH, TSH, and Prolactin was determined. Blood and follicular fluid were then collected for thiole-disulfide assay on the day of oocyte retrieval.

Inclusion criteria: Age 20-42 years old, BMI: 18-35 kg/m², Non-smoker, No systemic or endocrine diseases.

Exclusion criteria: Age above 42, Smoker, Women with systemic or endocine diseases, Women with a cut infection, Women using a vitamin supplement.

All patients underwent controlled ovarian stimulation using the antagonist protocol, beginning on day 2 of the cycle with gonadotropin (Gonal-F, Serono, Italy). Starting at 150-300 mIU/d, the dosage was modified according on age, body weight, and the number of antral follicles. Transvaginal ultrasonography was used to track follicular growth every two to three days from stimulation until ovulation. The gonadotropin dosages were modified based on the ovarian response and serum E2 level on day seven after stimulation. When the diameter of the leading follicles reached 14 mm, 0.25 mg of cetrorelix (Merck-Serono, Germany) was injected subcutaneously. This dosage was continued every day until the delivery of human chorionic gonadotropin (hCG). When at least three follicles grew to a diameter of more than 18 mm, recombinant hCG (Ovitrelle, 250 µg, Merck, UK) was injected subcutaneously into each patient. The blood E2 level was measured on the day that hCG was administered. Under the guidance of transvaginal ultrasonography, the ovum was extracted 35-36 hours after hCG was administered for 12 hours. Five milliliters of whole blood and ten milliliters of follicular fluid were taken, and the thiol disulfide homeostasis was determined for each specimen. Clear serum was obtained by centrifuging the collected blood in a 6 ml coagulation gel tube at 3000 rpm after it had been incubated for 30 hours at 37 °C. The serum was then collected in 5 ml Eppendorf tubes and kept at -60 °C until analysis was completed. Using a 17-G oocyte recovery set (Wallace, USA), 10 ml of the follicular fluid was carefully extracted from follicles that were 18 to 24 mm in diameter during oocyte retrieval. The follicular cells were then separated by centrifuging the follicular fluid at 3000 rpm, and the resulting fluid supernatant was collected in 5 ml eppendorf tubes and kept at -60 °C until analysis was completed. One to four hours following oocyte retrieval, sperm injection of oocytes was carried out. The percentage of successfully fertilized oocytes out of the total number of injected oocytes was used to calculate the fertilization rate 16-18 hours after insemination. After that, on the third day prior to embryo transfer (ET), the quality of the embryo was assessed during development. Approximately 48 hours (6-8 cell stages) following fertilization two- three embryos were transplanted. For up to 12 weeks of pregnancy, luteal phase support was started on the day of discovery with 400 mg Cyclogest vaginal suppositories placed twice daily for two weeks (Merck Actives, England). Two weeks following ET, a pregnancy test (Biochemical pregnancy) was performed. When one or more gestational sacs showed signs of heart activity, it was considered clinical pregnancy

Determinationation of serum and follicular dynamic Thiol/disulphide homeostasis

A- Priniciple of the Assay: Half of the difference between the total thiols and the native thiols was recorded as the dynamic disulphide amount. After the native thiols (SH) and total thiols were determined, disulphide (SS) amounts, disulphide/total thiol percent ratios (SS/SH+SS), disulphide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH+SS) were calculated [26].

B. Equations used in the calculation

- Native Thiol Status (-SH)
- Dynamic Disulfide Status (-S-S-)
- Total (Oxidized and Reduced) Thiol Status (-SH + -S-S-)
- Reduced Thiol Ratio [(-SH)/ (-SH + -S-S-)]X 100
- Oxidized Thiol (disulfide) Ratio [(-S-S-)/ (-SH + -S-S-)] X 100
- Thiol Oxidation Reduction Ratio [[(-SH)/ (-S-S-)]X 100

Determination of serum and follicular total thiols(-SH + -S-S-)

Priniciple of the Assay for total and native thiols: Reducible disulfide bonds were reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native thiol groups were determined after the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Half of the difference between the total thiols and the native thiols was recorded as the dynamic disulfide amount. After the native thiols (SH) and total thiols were determined, disulphide (SS) amounts, disulfide/total thiol percent ratios (SS/SH+SS), disulfide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH+SS) were calculated (27, 28, 30, 31).

Statistical analysis: The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010. The descriptive statistics including mean and standard error were measured to describe the data and the groups were compared by applying ANOVA (Analysis of variance test was used to compare more than two different groups) and chi square (Comparison between non continuous variables or percentages). The degree of association between continuous variables was calculated by Pearson's correlation coefficient (r) and the results were considered statistically significant when p value was equal to or less than 0.05.

Results

Classifications of the patients enrolled in the present study

Fifty infertile females were enrolled in the present study; the females were divided into 3 groups according to ovarian stimulation protocols into; normal responders (22 females), hyper responders (9 females) and poor responders (19 females) (Figure 1).

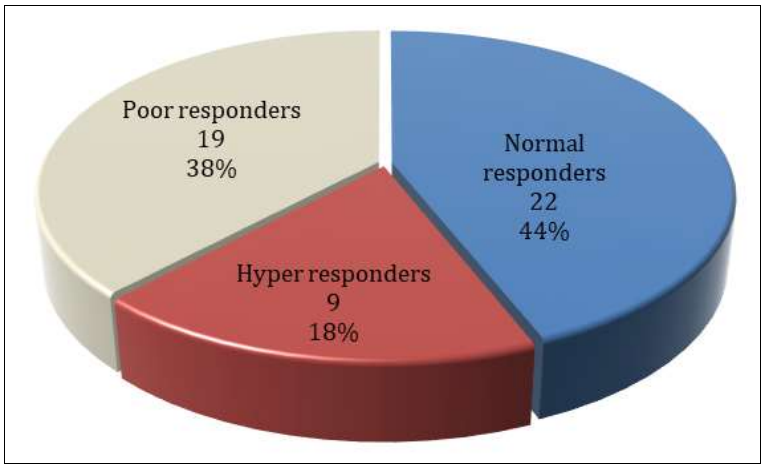

Fig 1: Classification of patients enrolled in the present study

Comparison of clinical data between the studied groups

Comparison of mean age, body mass index (BMI) and hormonal levels between the studied groups

The comparison of mean patients age, body mass indices and hormonal levels between normal, hyper and poor responders were demonstrated in table 2 and figure2, according to the results there were significant differences regarding patients ages (30.18±1.85 vs. 26.11±1.17vs.36.21±1.70; *p*=0.004) and AMH levels (2.36±0.24 vs. 5.95±0.49 vs. 1.04±0.18; *p*<0.001); however there were no significant differences between the studied groups concerning body mass indices (*p*=0.086) and FSH levels (*p*=0.188).

Table 2: Comparison of mean age, BMI and hormonal levels between the studied groups

Parameters	Normal responders N.=22 (Mean±SE)	Hyper responders N.=9 (Mean±SE)	Poor responders N.=19 (Mean±SE)	*p* value
Age (years)	30.18±1.85	26.11±1.17	36.21±1.70	0.004 ∀S
BMI (Kg/m²)	23.03±0.72	24.69±1.88	25.58±0.60	0.086 ∀NS
AMH (ng/ml)	2.36±0.24	5.95±0.49	1.04±0.18	<0.001 ∀S
FSH (mIU/ml)	6.83±0.87	5.21±0.38	14.13±2.27	0.188 ∀NS

SE: Standard error; BMI: Body mass index; FSH: Follicle stimulating hormone; AMH: Antimullerian hormone; S: Significant (*p*≤ 0.05); NS: Non significant (p>0.05); ∀: ANOVA t test

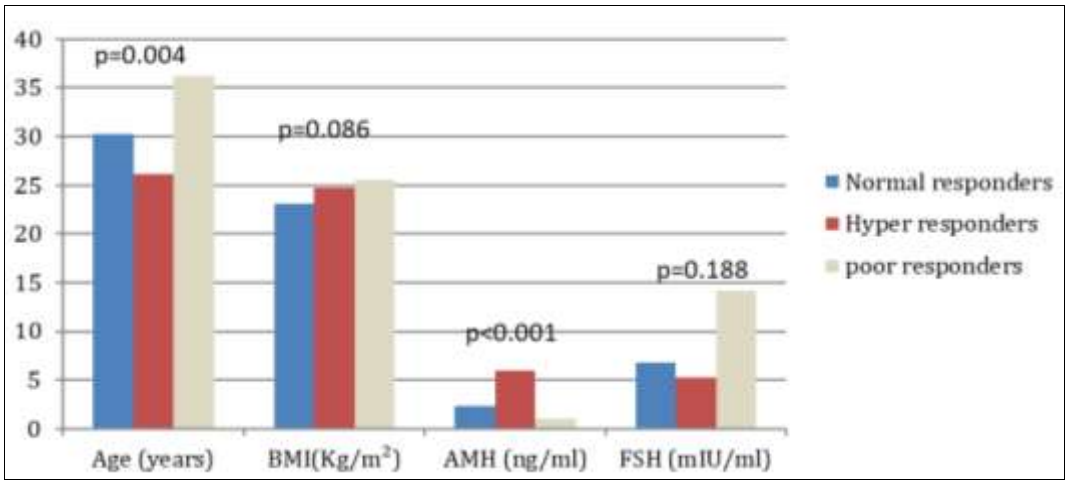

Fig 2: Comparison of mean age, BMI and hormonal levels between the studied groups

Comparison of ICSI outcomes between the studied groups

Total oocytes count, metaphase II oocytes and positive pregnancy rates were significantly higher among hyper responders; (11.23±0.97 vs. 21.22±1.75 vs. 5.47±1.08; *p*< 0.001), (9.05±0.78 vs. 15.00±1.52 vs. 4.00±0.95; *p*< 0.001) and (22.7% vs. 44.4% vs. 5.3%; *p*=0.044) respectively; on the other hand, transferred embryos count were significantly higher among normal responders (2.45±0.22 vs. 1.56±0.71vs.1.00±0.30; *p*=0.005); however there was no significant differences regarding fertilization rates between the studied groups (80.76±4.97 vs. 76.58±9.20 vs. 62.61±9.44; *p*=0.192) as illustrated in table 3, figure 3 and figure 4.

Table 3: Comparison of ICSI outcomes between the studied groups

ICSI parameters	Normal responders N.=22	Hyper responders N.=9	Poor responders N.=19	*p* value
Total oocytes count (Mean± SE)	11.23±0.97	21.22±1.75	5.47±1.08	< 0.001 ∀ S
Metaphase II oocytes (Mean± SE)	9.05±0.78	15.00±1.52	4.00±0.95	< 0.001 ∀ S
Fertilization rates (Mean± SE)	80.76±4.97	76.58±9.20	62.61±9.44	0.192 ∀ NS
Transferred embryos (Mean± SE)	2.45±0.22	1.56±0.71	1.00±0.30	0.005 ∀ S
Positive pregnancy rate N. (%)	5 (22.7%)	4 (44.4%)	1 (5.3%)	0.044 Ⅽ S

S: Significant ($p \leq 0.05$); NS: Non significant (p>0.05); ∀: ANOVA t test; Ⅽ: Chi square

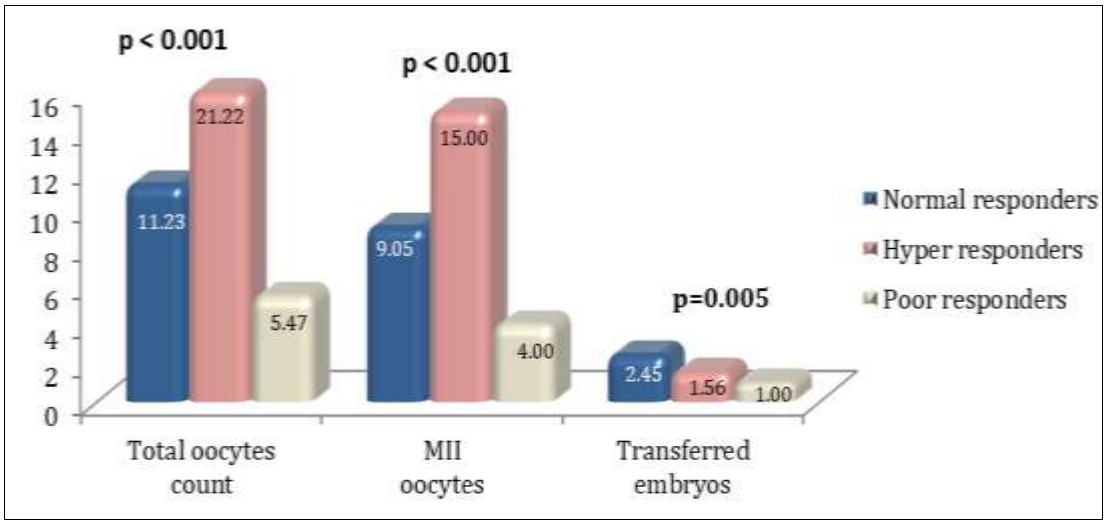

Fig 3: Comparison of total oocytes count, MII oocytes and transferred embryos between the studied groups.

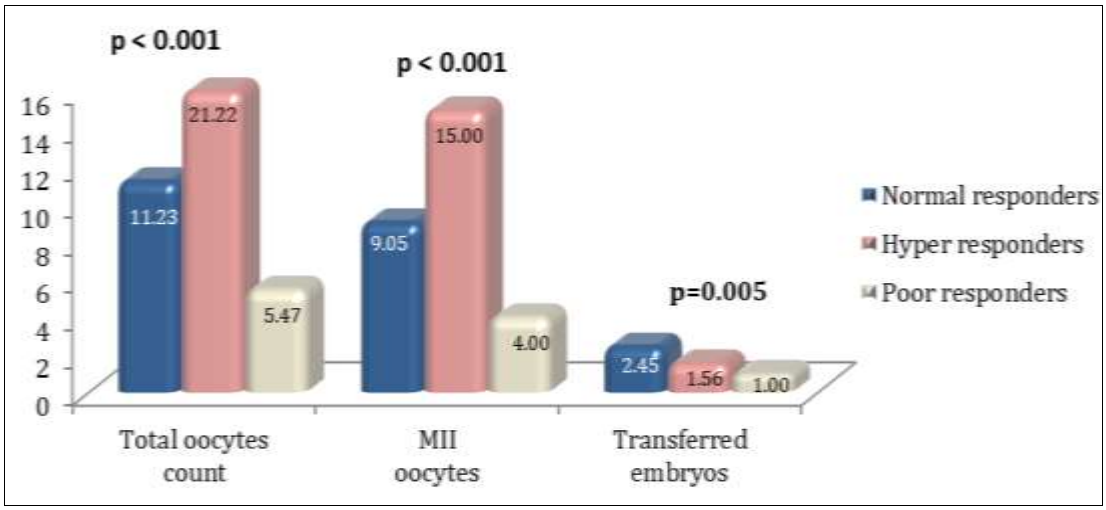

Fig 4: Comparison of fertilization and pregnancy rates between the studied groups

Comparison of Serum and Follicular Fluids Thiol / Disulfide Homeostasis Parameters between the Studied Groups

There were no significant differences between normal responders, hyper responders and poor responders concerning serum and follicular fluids thiol / disulfide homeostasis parameters as presented in table 4 and table 5.

Table 4: Comparison of serum thiol / disulfide homeostasis parameters between the studied groups.

Thiol-DS homeostasis	Normal responders N.=22 (Mean± SE)	Hyper responders N.=9 (Mean± SE)	Poor responders N.=19 (Mean± SE)	*p* value
Native thiol (µmol/l)	340.3±12.47	314.7±8.96	313.7±8.44	0.152 ∀ NS
Dynamic DS(µmol/l)	44.71±6.86	48.28±10.71	47.32±8.24	0.952 ∀ NS
Total thiol(µmol/l)	429.7±19.35	411.2±15.47	408.4±17.87	0.667 ∀ NS
Reduced thiol- DS ratio	80.56±2.52	77.68±4.29	78.73±2.29	0.810 ∀ NS
Oxidized thiol-DS ratio	9.72±1.26	11.16±2.15	10.63±1.46	0.810 ∀ NS
Thiol oxidation reduction ratio	1719±380	1148±336	1194±228	0.408 ∀ NS

NS: Non significant (p>0.05); DS: Disulfide; ∀: ANOVA t test

Table 5: Comparison of follicular fluids thiol / disulfide homeostasis parameters between the studied groups.

Thiol-DS homeostasis	Normal responders N.=22 (Mean± SE)	Hyper responders N.=9 (Mean± SE)	Poor responders N.=19 (Mean± SE)	*p* value
Native thiol (µmol/l)	243.3±17.81	252.7±42.19	253.2±23.71	0.942 ∀ NS
Dynamic DS (µmol/l)	22.52±3.94	33.61±5.20	23.36±5.84	0.376 ∀ NS
Total thiol- (µmol/l)	288.3±20.49	319.9±43.23	299.9±32.91	0.800 ∀ NS
Reduced thiol- DS ratio	84.77±2.22	76.99±4.38	85.77±1.79	0.092 ∀ NS
Oxidized thiol-DS ratio	7.62±1.11	11.50±2.19	7.11±0.89	0.092 ∀ NS
Thiol oxidation reduction ratio	1748±270	927±196	2190±638	0.265 ∀ NS

NS: Non significant (p>0.05); DS: Disulfide; ∀: ANOVA t test

Correlation between Serum and Follicular Fluids Thiol / Disulfide Homeostasis Parameters with ICSI Outcomes

*** Correlation between serum thiol / disulfide homeostasis parameters with ICSI outcomes**

There was a solitary significant positive correlation between transferred embryos count with thiol oxidation/reduction ratio (r=0.329 & *p*=0.020) (Figure 5); however there were no significant correlations between all thiol/disulfide homeostasis parameters with total oocytes, metaphase II oocytes and fertilization rates as demonstrated in Table 6.

Table 6: Correlation between serum thiol / disulfide homeostasis parameters with ICSI outcomes.

		Native thiol	Dynamic DS	Total thiol	Reduced thiol-DS ratio	Oxidized thiol-DS ratio	Thiol O-R ratio
Total oocytes count	r	-0.043	-0.129	-0.134	0.117	-0.117	0.121
	p value	0.768 NS	0.372 NS	0.354 NS	0.417 NS	0.417 NS	0.402 NS
Metaphase II oocytes	r	-0.012	-0.210	-0.183	0.209	-0.209	0.230
	p value	0.933 NS	0.143 NS	0.203 NS	0.144 NS	0.144 NS	0.108 NS
Fertilization rate	r	0.027	-0.218	-0.166	0.219	-0.219	0.224
	p value	0.855 NS	0.129 NS	0.249 NS	0.127 NS	0.127 NS	0.117 NS
Total embryos		0.219	-0.187	-0.025	0.245	-0.245	0.329
		0.127 NS	0.194 NS	0.865 NS	0.086 NS	0.086 NS	0.020 S

r: Pearson correlation coefficient; NS: Not significant (p> 0.05); S; Significant (*p*≤0.05); OR: Oxidation reduction.

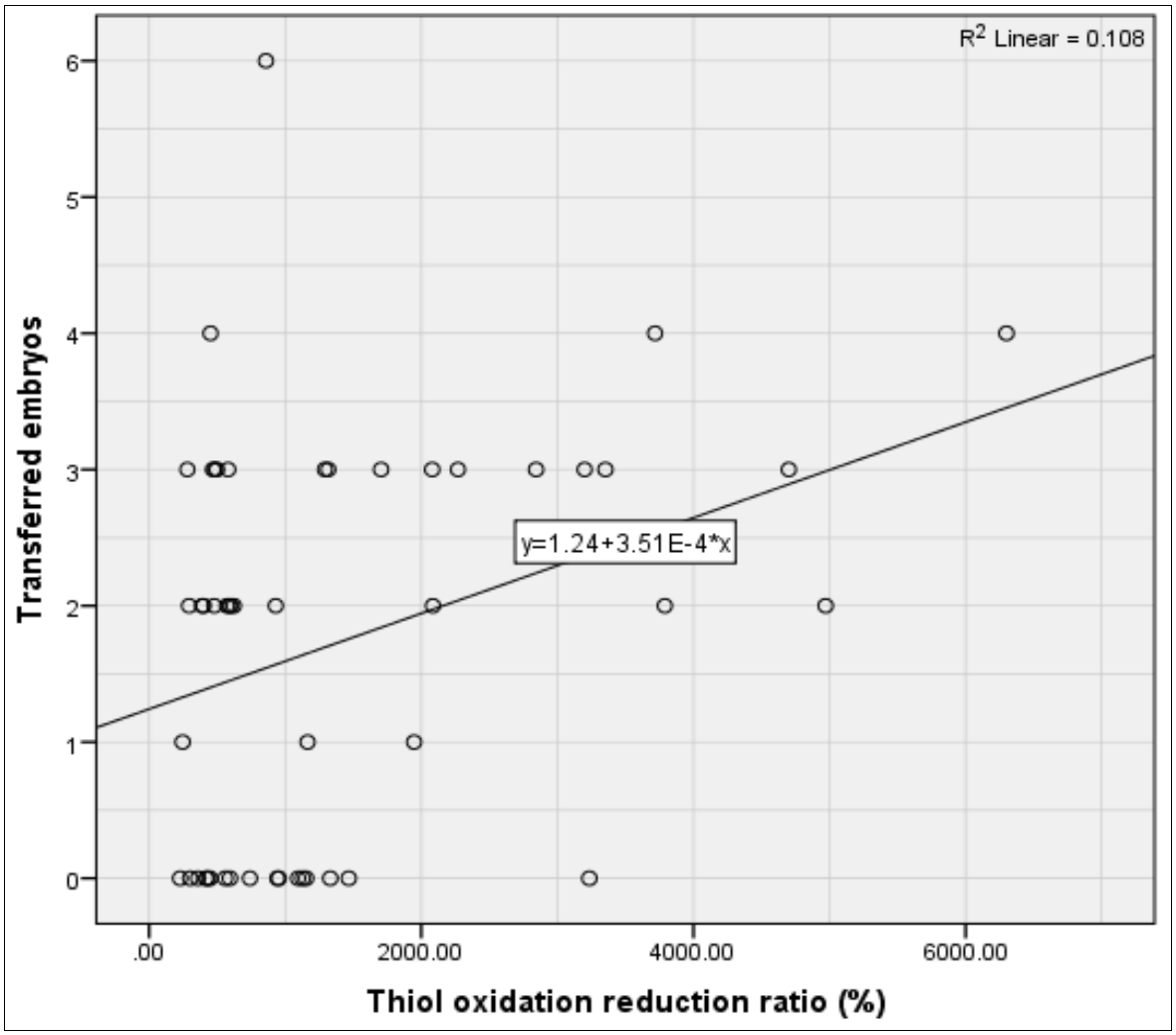

Fig 5: Correlation between serum thiol oxidation reduction ratio with transferred embryos.

***Correlation Between Follicular Fluids Thiol / Disulfide Homeostasis Parameters With ICSI Outcomes.**

Correlations between follicular fluids thiol / disulfide homeostasis parameters with ICSI outcomes were illustrated in table 7, according to the results there were no significant differences between all thiol disulfide parameters with total oocytes, metaphase II oocytes, fertilization rates and transferred embryos

Table 7: Correlation between serum and follicular fluids thiol / disulfide homeostasis parameters.

		Native thiol	Dynamic-DS	Total thiol	Reduced thiol-DS ratio	Oxidized thiol-DS ratio	Thiol O-R ratio
Total oocytes count	r	-0.009	-0.049	-0.024	-0.063	0.063	-0.103
	p value	0.954 NS	0.736 NS	0.869 NS	0.666 NS	0.666 NS	0.481 NS
Metaphase II oocytes	r	-0.027	-0.135	-0.069	0.028	-0.028	0.133
	p value	0.854 NS	0.354 NS	0.635 NS	0.848 NS	0.848 NS	0.364 NS
Fertilization rate	r	0.039	-0.148	-0.020	0.087	-0.087	-0.042
	p value	0.792 NS	0.309 NS	0.890 NS	0.551 NS	0.551 NS	0.777 NS
Transferred embryos	r	-0.238	-0.001	-0.195	-0.220	0.220	-0.239
	p value	0.099 NS	0.996 NS	0.179 NS	0.129 NS	0.129 NS	0.098 NS

r: Pearson correlation coefficient; NS: Not significant (p> 0.05); OR: Oxidation reduction

Discussion

This study revealed that there were substantial differences between the study groups in terms of patient ages and AMH levels, but no significant variations in body mass indices. The total oocyte count, metaphase II oocytes, and positive pregnancy rates were significantly higher among hyperresponders. The pregnancy rate was not included in other statistics since the majority of embryos were frozen in hyperresponders and banked in poor responders. On the other hand, the number of transplanted embryos was much higher among normal responders because most were transferred fresh, whereas the majority of hyperresponders were frozen; yet, there were no significant variations in fertilization rates across the study groups. The thiol/disulfide homeostasis parameters of serum and follicular fluids did not significantly differ among normal responders, hyperresponders, and poor responders. Total oocytes, metaphase II oocytes, and fertilization rates did not significantly correlate with any of the thiol/disulfide homeostasis markers.

Tola *et al.* [22] examined follicular fluid thiol/disulfide homeostasis in PCOS and found a positive correlation between higher native thiol levels and the rate of fertilization in polycystic ovaries. This contrasts with our study, which found no relationship with patients who had hyperresponders, the majority of whom had PCO. This discrepancy may have resulted from our study's small sample size. In line with our findings that M2 had no relationship to thiol/disulfide homeostasis, Ayse Z. Ozdemir *et al.* [23] conclude that a statistically significant correlation was found between the isolated disulfide level and M2 athiol/disulfide homeostasis, which did not differ significantly. This indicates that a small amount of OS is required for oocyte maturation. Kadriye Erdoğan *et al.* [24] found that DOR patients had lower serum and FF total and native thiol levels, which contradicts our findings of no difference in all groups. This could be due to differences in patient samples, including only Poseidon 3 in their study versus all Poseidon groups, as well as differences in stimulation protocols, which may impact the antioxidant role in the body.

Similar to our study, the serum and FF disulfide levels, as well as the serum and FF disulfide/native thiol ratios, which indicate the state of OS, were not higher in DOR patients compared to the control group. This could indicate that OS has little to no impact on the follicular fluid microenvironment of oocytes.

Esengul Türkyılmaz [25], who evaluated the follicular fluid thiol/disulfide balance among patients with poor ovarian response, found that disulfide levels were significantly different between the NOR and POR groups. The NOR group's higher disulfide levels may have contributed to the statistically significant differences in the number of metaphase II oocytes and the percentage of good-quality embryos compared to the POR group, and the total thiol levels were correlated with the total number of 2PN oocytes. This is in contrast to our study, which found no correlation between thiol and disulfide in both normal and poor responders and the number of metaphase II oocytes. Moreover, concur with our research that oxidative markers disulfide and antioxidant thiol play no part in poor responders. This can be explained by the negative effects of disulfide, which may have accumulated as an environmental toxin that affects the normal group.

Conclusion

We can conclude that there are no significant correlations between the antioxidant markers total thiol and native thiol and oxidant marker disulfide in serum and follicular fluid and normal, hyper, and poor responders. Further investigation is required to determine how these relationships evolve.

Recommendation

OS is a significant problem in human reproduction. There are numerous studies about it in the literature. Thiol/disulfide homeostasis is a less expensive approach of demonstrating OS. More research is needed to understand and elucidate the impacts of OS. It is believed that new medicines may be discovered to improve IVF success by resolving the OS problem.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article
Bakir NA, Al-Ghazali BS, Shakir N, Abbood MS. Association of serum and follicular fluid thiol / disulfide homeostasis in patients undergoing intracytoplasmic sperm injection. International Journal of Clinical Obstetrics and Gynaecology. 2025; 9(1): 156-163.