



World Journal of Current Medical and Pharmaceutical Research

Content available at www.saap.org.in

ISSN: 2582-0222



DYNAMIC THIOL/DISULFIDE HOMEOSTASIS AS AN OXIDATIVE STRESS BIOMARKER IN MALE AND FEMALE INFERTILITY AND ASSISTED REPRODUCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

DURAR KAMIL JABBAR* 

Department of Basic Sciences, College of Dentistry, University of Al-Qadisiyah, Al-Diwaniyah, Iraq.

DOI: <https://doi.org/10.37022/wjcmpr.v8i3.442>

ARTICLE HISTORY	ABSTRACT
Received on: 19-07-2026 Revised on: 14-09-2026 Accepted on: 03-10-2026	Oxidative stress is often blamed for infertility, yet most redox markers used in reproductive research are hard to compare between laboratories. Dynamic thiol/disulfide homeostasis (TDH), measured with the automated assay of Erel and Neşelioğlu, gives native thiol (NT), total thiol (TT), disulfide (SS) and three ratio indices from a single sample. We systematically reviewed human studies that used this assay in male or female infertility and in assisted reproduction. Web-engine, Crossref and DOAJ searches with citation chasing yielded 204 records, and 28 studies met the criteria (23 in women, 5 in men). All but one came from Turkey. Thirteen studies had usable group data and were pooled with random-effects models (REML, Hartung-Knapp). In the serum or plasma of infertile women, absolute NT, TT and SS did not differ significantly from those of controls, and heterogeneity was high. The ratio indices were more consistent. SS/NT (Hedges' g 0.34, 95% CI 0.06 to 0.62), SS/TT (g 0.53, 0.11 to 0.94) and NT/TT (g -0.54, -1.03 to -0.05) all moved toward oxidation, and 14 of 15 studies showed a higher SS/NT. Follicular fluid estimates were imprecise. The five male studies, on varicocele and idiopathic infertility, reported only oxidative shifts but could not be pooled. The signal was weak. It faded when three studies with atypical absolute values were removed, and the certainty of evidence was very low. TDH ratios are promising research markers, but assay standardization and larger, better designed studies are needed before clinical use.
Keywords: Thiol/disulfide homeostasis; native thiol; disulfide; oxidative stress; female infertility; male infertility; polycystic ovary syndrome; varicocele; assisted reproductive technology	
Corresponding Author Durar Kamil Jabbar	

This article is licensed under a Creative Commons Attribution-Non-commercial 4.0 International License.
Copyright © 2026 Author(s) retains the copyright of this article.



INTRODUCTION

Almost one in six people of reproductive age will experience infertility at some point in their lives [1]. The causes are common to the spouses. On the female side, ovulatory abnormalities, endometriosis, tubal disease, low ovarian reserve are prevailing, whereas varicocele and unexplained semen defects are common in men [2,3]. But for many couples the work-up stops with no evident mechanism. One theory that keeps popping up is oxidative stress. Gametes require tiny levels of reactive oxygen species (ROS) for signalling, capacitation and ovulation, but an excess can damage lipids, proteins and DNA [4,5]. The modern perspective is that oxidative stress is less a mere excess of oxidants and rather a lack of redox regulation [6-8]. That control is centred around thiol groups. Cysteine residues are sensitive recorders of redox stress [9], switching between the reduced (-SH) and the oxidised (S-S-) state.

In 2014, Erel and Neşelioğlu introduced a completely automated assay for the measurement of native thiol, total thiol and dynamic disulphide in the same run [10]. The test

is cheap and rapid and is run on regular clinical chemical analysers, therefore it spread swiftly, above all in Turkey [11]. Reproductive researchers soon applied it to polycystic ovary syndrome (PCOS), endometriosis, ovarian insufficiency, male infertility and in vitro fertilization (IVF). The results point in different directions. Some studies report a clear shift toward disulfide formation, others find no change, and a few find the opposite. As far as we know, no review has pooled these data across male and female infertility. We therefore asked a simple question. Does dynamic thiol/disulfide homeostasis (TDH) differ between people with infertility-related conditions and suitable reference groups, and does it relate to the outcome of assisted reproduction?

THE DYNAMIC THIOL/DISULFIDE SYSTEM

Thiol pools in plasma and reproductive fluids

Plasma thiols are mostly bound to protein. Albumin carries one free cysteine, Cys34, and this single residue holds the largest share of the reduced thiol pool [12,13]. Low-molecular-weight thiols such as cysteine, homocysteine, cysteinylglycine and glutathione are present at much lower levels, but they exchange quickly with protein thiols [12].

When oxidants attack, two thiols can join into a disulfide, either between two small molecules (cystine, glutathione disulfide) or as a mixed disulfide with a protein (Figure 1a). Thioredoxin and glutaredoxin, fed by NADPH, reverse many of these bonds. The system is therefore dynamic, and its steady state tells us something about the oxidant load that the body is handling [9,11].

Reproductive fluids draw on the same chemistry. Follicular fluid is largely a plasma transudate, modified by the secretions of granulosa and theca cells, and it bathes the oocyte during its final maturation [14]. Seminal plasma protects spermatozoa after ejaculation, because sperm have little cytoplasm and few antioxidant enzymes of their own [5]. Disulfides are not always a sign of damage, however. During epididymal transit, protamine cysteines form disulfide bridges that compact the sperm nucleus, and this is a normal step of maturation [15-17]. What matters is the balance, and where in the body it is measured.

The automated assay and its indices

The Erel method splits the sample into two reactions (Figure 1b). In the first, native thiols react directly with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent), which releases a yellow anion read near 412 nm [18]. In the second, sodium borohydride reduces the dynamic disulfides back to thiols, formaldehyde removes the leftover reductant, and DTNB then measures total thiol [10]. Half of the difference between total and native thiol is the disulfide amount. Three ratios follow, each multiplied by 100: SS/NT, SS/TT and NT/TT. Because NT/TT plus twice SS/TT equals 100%, the three ratios are linked and should not be read as independent evidence.

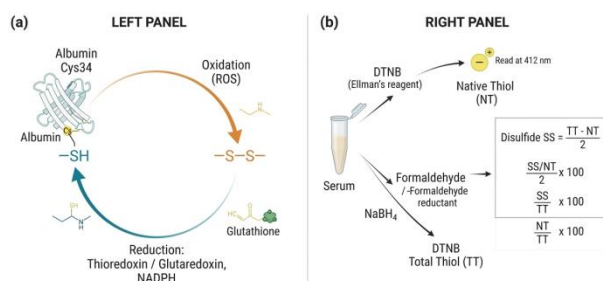


Figure 01: The dynamic thiol/disulfide system. (a) Reduced thiols in plasma, dominated by albumin Cys34, are oxidized to disulfides and reduced back by thiol-reducing systems.

(b) Principle of the automated assay: native thiol is read directly with DTNB, total thiol is read after borohydride reduction and removal of the excess reductant with formaldehyde, and disulfide and the three indices are calculated.

Sample handling matters a great deal. In serum, TDH stayed stable for three months at -20°C and for twelve months at -80°C , while longer storage at -20°C and repeated freeze-thaw cycles lowered native and total thiol and raised disulfide [19]. Albumin level and body weight also move the absolute values, as several of the included studies showed. These points become important once data from different laboratories are combined.

METHODS

Protocol, information sources and search

The review follows the PRISMA 2020 statement [20]. It was not registered in advance, which we list as a limitation. The final searches were run on 29 September 2026 with no start date. Sources were a general web search engine (with

site-restricted queries on DergiPark and publisher platforms), the Crossref and DOAJ bibliographic interfaces, single-record look-ups in OpenAlex and Semantic Scholar, and forward citation chasing through OpenCitations. Automated querying of PubMed, PubMed Central and Europe PMC was not possible during the search period (captcha pages and rate limits), so records indexed there were reached through the other routes. Search strings combined thiol/disulfide, thiol-disulfide, thiol/disulphide, disulphide homeostasis, native thiol and dynamic thiol with clinical terms. These covered PCOS, endometriosis, ovarian reserve, ovarian insufficiency, unexplained infertility, follicular fluid, IVF, ICSI, embryo quality, pregnancy, semen, sperm, varicocele and male infertility. The Turkish words tiyol and disülfit were searched as well.

Eligibility criteria

We included human studies of any design that measured TDH with the automated Erel-Neşelioğlu method or with the same borohydride/DTNB chemistry. Participants had to have infertility or an infertility-related diagnosis, such as PCOS, endometriosis, diminished ovarian reserve or poor ovarian response, premature ovarian insufficiency, unexplained infertility, varicocele or idiopathic male infertility, or they had to be undergoing assisted reproduction. Each study needed a comparison: a reference group, outcome-defined groups within a treatment cycle, or values before and after treatment. Serum, plasma, follicular fluid and seminal plasma were all eligible. We excluded animal and in vitro studies, reviews, studies that measured only free or protein thiols with other methods, and pregnancy complications. Recurrent pregnancy loss was left out of the synthesis but is mentioned in the Discussion. Congress abstracts were kept when they reported data or the direction of effect.

Selection, data extraction and quality appraisal

Records were screened in four parallel streams (PCOS, other female infertility, assisted reproduction and male infertility), and the logs were then merged and de-duplicated by DOI and title. Group sizes, means and standard deviations (or medians with interquartile ranges, or standard errors) were copied from the tables of the full texts. A second, independent extraction of all 14 quantitative sources gave identical values. When only the abstract could be read, we recorded the direction and significance of each reported difference. Two reporting errors needed a decision. One study printed a control standard deviation for native thiol that equaled the control median ($451.8 \pm 428.5 \mu\text{mol/L}$), so this value was left out of the primary analysis and tested in sensitivity analyses [21]. In another, the two disulfide ratios were transposed, since SS/NT cannot be smaller than SS/TT, and we re-assigned them [22].

The 14 quantitative studies were appraised with a seven-item checklist adapted from the Newcastle-Ottawa Scale [23]. The items were an explicit case definition, a defined reference group, comparable age, comparable body mass index (BMI), exclusion of smokers and of drug or antioxidant users, standardized sampling with storage at -80°C , and blinded laboratory analysis. Items that were not reported were scored as not met. The certainty of each pooled estimate was rated with GRADE [24].

Statistical analysis

The effect measure was the standardized mean difference (Hedges' g), condition minus reference [25], because absolute concentrations differed several-fold between laboratories. Medians with interquartile ranges were converted to means and standard deviations with the methods of Luo et al. and Wan et al. [26,27], and standard errors with $SD = SE \times \sqrt{n}$. Random-effects models were fitted with a restricted maximum likelihood estimate of τ^2 , and 95% confidence intervals (CI) were built with the Hartung-Knapp-Sidik-Jonkman method and its ad hoc variance correction [28,29]. Heterogeneity was described with I^2 [30], and 95% prediction intervals (PI) were added when at least three studies were pooled [31]. Circulating (serum or plasma) and follicular fluid data were analyzed separately, with PCOS and non-PCOS subgroups. Sensitivity analyses used the DerSimonian-Laird estimator with Wald intervals [32], leave-one-out runs, removal of studies with atypical absolute values or with converted statistics, and restriction to studies with a quality score of at least 5 of 7. Egger's test was applied when ten or more studies were available [33]. Because many studies could only be read as abstracts, we also counted the direction of effect for all case-reference comparisons (one per study). A two-sided sign test was applied and an effect direction plot was drawn, following the SWiM guideline [34,35]. Analyses were run in Python (NumPy and SciPy) and checked against the pymare and statsmodels libraries.

RESULTS

Study selection and characteristics

The searches yielded 204 unique records (Figure 2). After screening of titles and abstracts, 67 reports were assessed in detail and 39 were excluded, most often because another thiol method had been used ($n = 21$). Twenty-eight studies published between 2016 and 2026 were included (Table 1) [21,22,36-61]. All of them came from Turkey except one ICSI study from Iraq [56]. Twenty-three studies involved women: 12 on PCOS, three on endometriosis, two on premature ovarian insufficiency, two on unexplained infertility, and four on ovarian reserve, ovarian response or IVF outcome. Five studies involved men with varicocele or idiopathic infertility. Together they reported on about 2,200 participants, although one study did not give its group sizes [37]. Full texts could be read for 14 studies. For the other 14, including all five male studies, only the abstract was accessible. Thirteen studies entered at least one meta-analysis: 11 in serum or plasma and four in follicular fluid, two of which contributed to both [54,56].

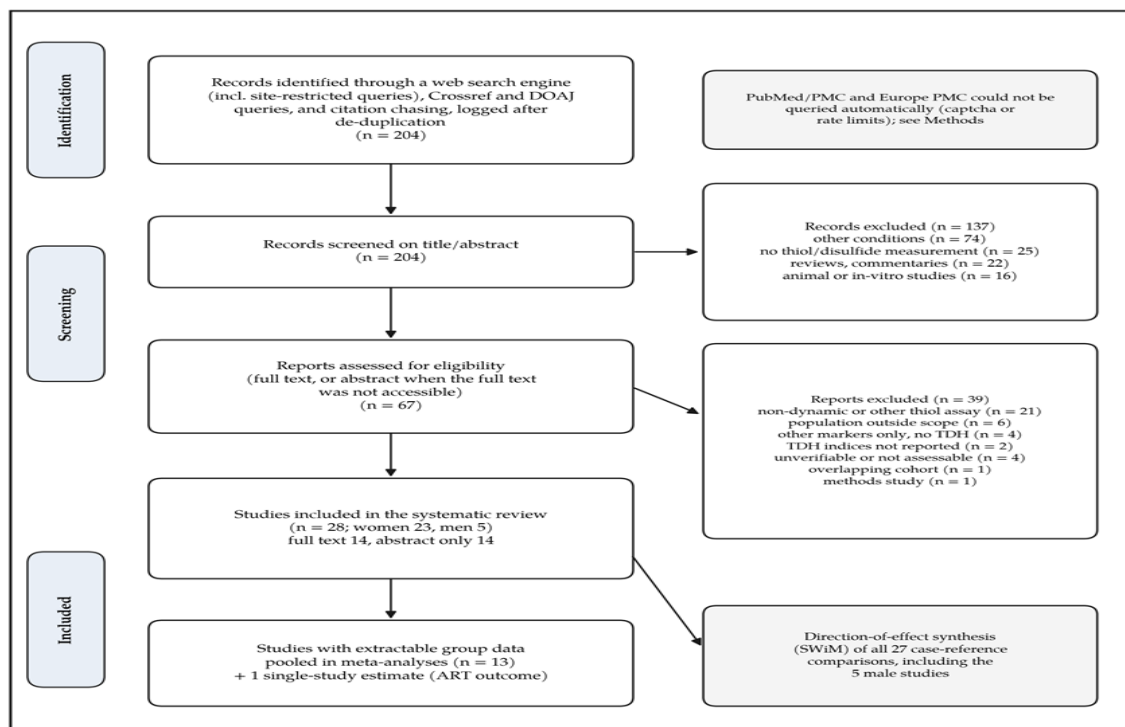


Figure 02: PRISMA 2020 flow diagram of the search and selection process.

Table 01: Characteristics of the 28 included studies.

Study	Condition and comparison (case vs reference)	Sample	n (case/ref)	Data	Main thiol/disulfide findings (case vs reference)
Polycystic ovary syndrome					
Ozler 2016 [36]	Overweight PCOS vs normal-weight PCOS and controls (adolescents)	S	43/45/96	A	NT, TT lower in overweight PCOS
Yildirim 2017 [37]	Obese and non-obese PCOS vs BMI-matched controls	S	NR	A	Thiol higher, SS lower in PCOS

Study	Condition and comparison (case vs reference)	Sample	n (case/ref)	Data	Main thiol/disulfide findings (case vs reference)
Sevimli 2017 [38]	PCOS vs controls (congress abstract)	S	49/44	A	NT, TT lower; SS, SS/NT, SS/TT higher
Tola 2018 [39]	PCOS vs tubal-factor IVF patients	FF	22/20	F	NT, NT/TT lower; SS, SS/NT, SS/TT higher; NT correlated with fertilization rate
Aydın 2020 [40]	PCOS vs controls	S	36/24	F	No significant difference
Ege 2021 [41]	Clomiphene-resistant vs clomiphene-sensitive infertile PCOS	S	30/30	A	SS higher; NT, TT and indices differed after adjustment
Biyik 2021 [21]	Non-obese PCOS vs age- and BMI-matched controls	S	44/44	F	NT lower; other parameters not significant
Laleli 2021 [42]	Adolescent PCOS vs healthy adolescents	S	30/30	F	No significant difference; thiols lower with overweight and higher CRP
Songur Dağlı 2022 [43]	Obese vs non-obese PCOS	S	41/37	A	NT, TT differed by obesity; thiols fell as BMI rose
Sen 2024 [44]	PCOS vs controls	S	30/30	F	NT, TT, NT/TT lower; SS, SS/NT, SS/TT higher
Aygün Arı 2025 [45]	Adolescent PCOS vs age- and BMI-matched controls	S	33/43	F	No significant difference; thiols fell with BMI and CRP
Horasanlı 2026 [46]	Infertile PCOS vs fertile women	S	20/20	F	SS higher; other parameters not significant
Endometriosis					
Turkyilmaz 2016 [47]	Endometriosis vs laparoscopy controls	S	31/27	A	NT, TT lower
Atalay Mert 2022 [22]	Endometrioma vs tubal-ligation controls	P	40/40	F	NT, TT, NT/TT lower; SS/NT, SS/TT higher
Topbas Selcuki 2022 [48]	Endometriosis or endometrioma vs gynecology outpatients	S	86/60	F	No significant difference
Premature ovarian insufficiency					
Isik 2017 [49]	Premature ovarian failure vs controls	P	40/37	A	SS, SS/NT, SS/TT higher; NT/TT lower
Bozkaya 2020 [50]	Premature ovarian insufficiency vs controls	P	34/35	A	NT, TT lower; both fell as carotid intima-media thickness rose
Unexplained infertility					
Aksin 2025 [51]	Unexplained infertility vs fertile women	S	41/41	A	No significant difference
Savaş 2025 [52]	Unexplained infertility vs parous women	S	23/27	F	NT, NT/TT lower; SS, SS/TT higher
Ovarian reserve, ovarian response and IVF outcome					
Ozdemir 2020 [53]	IVF: not pregnant vs pregnant	S	40/22	F	No difference; SS correlated with metaphase II oocytes
Erdoğan 2023 [54]	Diminished ovarian reserve (POSEIDON 3) vs partners of male-factor couples, ICSI	S, FF	40/30	F	NT, TT lower in serum and FF; FF NT correlated with blastocyst score
Türkyilmaz 2025 [55]	Poor vs normal responders, ICSI	FF	27/35	F	SS lower in poor responders
Bakir 2025 [56]	Poor vs normal (and hyper-) responders, ICSI; Iraq	S, FF	19/22	F	No significant difference

Study	Condition and comparison (case vs reference)	Sample	n (case/ref)	Data	Main thiol/disulfide findings (case vs reference)
Male infertility					
Gul 2018 [57]	Infertile varicocele vs fertile varicocele and fertile controls	P	46/70/37	A	SS, SS/NT, SS/TT higher in infertile varicocele
Sonmez 2018 [58]	Varicocele with abnormal vs normal semen	Blood	92/25	A	SS and both SS ratios differed; SS related to poorer semen
Coban 2019 [59]	Spermatic vs peripheral vein blood in varicocele	Blood	61 paired	A	NT, TT, NT/TT lower and SS ratios higher in spermatic vein
Gok 2020 [60]	Before vs after varicocelectomy	Blood	56 paired	A	NT, TT rose after surgery; rise tracked semen changes
Micoogullari 2022 [61]	Idiopathic male infertility vs healthy men	S	79/90	A	NT, TT lower; SS/NT, SS/TT higher; NT cut-off 507 $\mu\text{mol/L}$

All studies were from Turkey except Bakir 2025 (Iraq). S, serum; P, plasma; FF, follicular fluid; Blood, sample type not stated in the abstract; F, group data from the full text; A, abstract only; NR, not reported; NT, native thiol; TT, total thiol; SS, disulfide; CRP, C-reactive protein; BMI, body mass index.

Quality scores ranged from 1 to 6 of 7, with a median of 4.5 (Table 2). Most studies used accepted diagnostic criteria, such as the Rotterdam criteria for PCOS [62], and most stored samples at -80°C . None reported blinding of laboratory staff. Reference groups were often weakly defined. Several were outpatients or infertile women with another diagnosis rather than women of proven fertility, and six of the 14 quantitative studies did not report BMI.

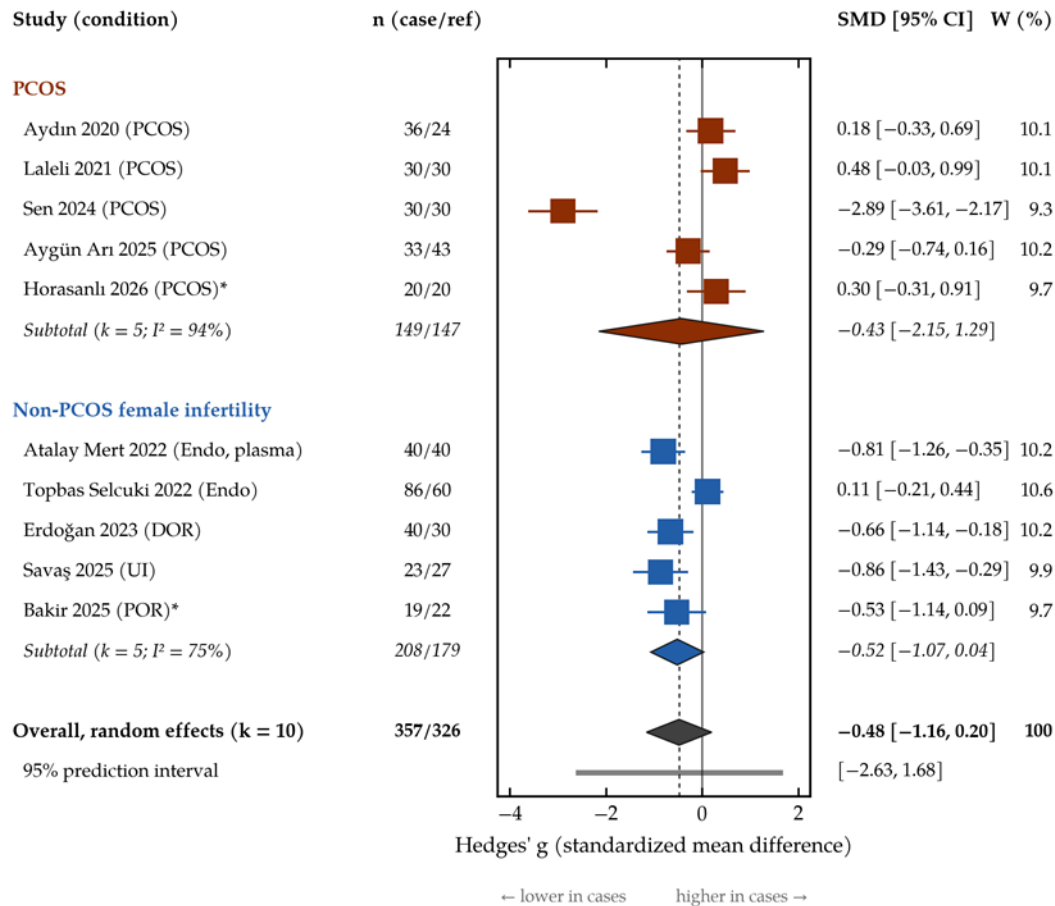
Table 02; Quality appraisal of the 14 studies with extractable group data (checklist adapted from the Newcastle-Ottawa Scale).

Study	Case definition	Reference group	Age	BMI	Smokers, drug users excluded	Standard sampling, -80°C	Blinding	Score (of 7)
Tola 2018 [39]	+	+	+	+	+	+	-	6
Ozdemir 2020 [53]	+	+	-	-	-	+	-	3
Aydin 2020 [40]	+	+	+	+	-	+	-	5
Biyik 2021 [21]	+	+	+	+	-	+	-	5
Laleli 2021 [42]	+	-	-	-	-	-	-	1
Atalay Mert 2022 [22]	+	+	+	-	-	+	-	4
Topbas Selcuki 2022 [48]	+	-	+	-	+	-	-	3
Erdoğan 2023 [54]	+	+	+	+	+	+	-	6
Sen 2024 [44]	+	+	-	-	-	-	-	2
Aygün Arı 2025 [45]	+	+	+	+	+	+	-	6
Savaş 2025 [52]	+	+	+	-	+	+	-	5
Türkyılmaz 2025 [55]	-	+	+	+	-	+	-	4
Bakir 2025 [56]	+	+	-	+	-	-	-	3
Horasanlı 2026 [46]	+	+	+	+	+	+	-	6

+ criterion met; - criterion not met or not reported. Age and BMI: matched or comparable between groups. Standard sampling means fasting or fixed cycle-day blood (or oocyte pick-up day, or blood-free follicular fluid) with storage at -80°C . Poor ovarian response in Türkyılmaz 2025 was defined only by oocyte yield. Bakir 2025 stored samples at -60°C .

Circulating thiol/disulfide homeostasis in female infertility

Absolute concentrations did not differ significantly between infertile women and reference groups (Table 3 and Figure 3; Supplementary Figures S1 to S6). Native thiol was lower (g -0.48 , 95% CI -1.16 to 0.20 ; $k = 10$; $I^2 = 89\%$) and so was total thiol (g -0.36 , -0.83 to 0.12 ; $k = 11$; $I^2 = 84\%$). Disulfide was higher (g 0.21 , -0.16 to 0.57 ; $k = 11$; $I^2 = 72\%$). None of these differences reached significance. The spread between studies was very wide. Sen et al. found a steep fall in native thiol in PCOS (g -2.89), whereas two other PCOS studies, one of them in adolescents, found slightly higher values than in controls [40,42,44].



Native thiol (circulating). Random-effects model (REML τ^2 , Hartung-Knapp-Sidik-Jonkman CI). Heterogeneity: $\tau^2 = 0.79$; $I^2 = 89\%$; $Q = 83.2$ (df = 9, $p < 0.001$). Test for overall effect: $p = 0.146$. Subgroup difference (PCOS vs non-PCOS): $Q = 0.02$, $p = 0.894$. Cases = condition group; ref = reference group. *Mean and SD estimated from median (IQR) or SE.

Figure 03: Forest plot of native thiol in the serum or plasma of women with infertility-related conditions versus reference groups. Squares are study estimates (size proportional to weight), diamonds are pooled estimates, and the grey bar is the 95% prediction interval. Endo, endometriosis; UI, unexplained infertility; DOR, diminished ovarian reserve; POR, poor ovarian response.

Table 03: Pooled standardized mean differences (condition minus reference), heterogeneity and certainty of evidence.

Parameter	Group	k	n (case/ref)	Hedges' g [95% CI]	p	I ² (%)	95% PI	Certainty
Native thiol	All women, serum/plasma	10	357/326	-0.48 [-1.16, 0.20]	0.146	89	-2.63 to 1.68	Very low
	PCOS, serum	5	149/147	-0.43 [-2.15, 1.29]	0.525	94	-5.12 to 4.26	
	Non-PCOS, serum/plasma	5	208/179	-0.52 [-1.07, 0.04]	0.060	75	-1.84 to 0.81	
Total thiol	Follicular fluid	4	108/107	-0.44 [-1.01, 0.13]	0.091	38	-1.62 to 0.74	Very low
	All women, serum/plasma	11	401/370	-0.36 [-0.83, 0.12]	0.125	84	-1.89 to 1.17	Very low
	PCOS, serum	6	193/191	-0.29 [-1.29, 0.70]	0.485	90	-3.00 to 2.41	
	Non-PCOS, serum/plasma	5	208/179	-0.43 [-0.94, 0.09]	0.085	71	-1.66 to 0.80	
Disulfide	Follicular fluid	4	108/107	-0.42 [-0.93, 0.09]	0.080	25	-1.34 to 0.50	Very low
	All women, serum/plasma	11	401/370	0.21 [-0.16, 0.57]	0.235	72	-0.86 to 1.27	Very low
	PCOS, serum	6	193/191	0.12 [-0.23, 0.47]	0.420	40	-0.53 to 0.77	
	Non-PCOS, serum/plasma	5	208/179	0.31 [-0.65, 1.27]	0.422	85	-2.19 to 2.80	

Parameter	Group	k	n (case/ref)	Hedges' g [95% CI]	p	I ² (%)	95% PI	Certainty
	Follicular fluid	4	108/107	0.23 [-1.22, 1.69]	0.645	88	-3.94 to 4.41	Very low
SS/NT	All women, serum/plasma	7	243/231	0.34 [0.06, 0.62]	0.025	32	-0.19 to 0.87	Very low
	PCOS, serum	5	163/161	0.34 [-0.01, 0.70]	0.056	24	-0.27 to 0.96	
	Non-PCOS, serum/plasma	2	80/70	0.32 [-3.66, 4.29]	0.495	72	n/a	
	Follicular fluid	3	89/85	0.47 [-1.65, 2.59]	0.442	88	-11.44 to 12.38	Very low
SS/TT	All women, serum/plasma	8	245/250	0.53 [0.11, 0.94]	0.021	71	-0.58 to 1.63	Very low
	PCOS, serum	5	163/161	0.47 [-0.23, 1.18]	0.135	78	-1.30 to 2.24	
	Non-PCOS, serum/plasma	3	82/89	0.63 [-0.50, 1.76]	0.139	59	-4.75 to 6.01	
	Follicular fluid	3	68/77	0.32 [-2.05, 2.68]	0.624	89	-13.01 to 13.64	Very low
NT/TT	All women, serum/plasma	7	201/206	-0.54 [-1.03, -0.05]	0.036	73	-1.82 to 0.74	Very low
	PCOS, serum	4	119/117	-0.49 [-1.52, 0.54]	0.226	82	-3.38 to 2.39	
	Non-PCOS, serum/plasma	3	82/89	-0.62 [-1.72, 0.49]	0.139	57	-5.85 to 4.61	
	Follicular fluid	3	68/77	-0.34 [-2.79, 2.12]	0.614	90	-14.24 to 13.56	Very low

Random-effects model with REML τ^2 and Hartung-Knapp-Sidik-Jonkman 95% CI; bold, CI excludes zero. Negative g for native thiol, total thiol and NT/TT, and positive g for disulfide, SS/NT and SS/TT, indicate a shift toward oxidation. PI, prediction interval ($k \geq 3$); n/a, not applicable. Certainty (GRADE) started at low for observational data and was downgraded for risk of bias, inconsistency and imprecision.

The ratio indices were more consistent. SS/NT was higher in infertile women (g 0.34, 95% CI 0.06 to 0.62; $k = 7$; $I^2 = 32\%$), and so was SS/TT (g 0.53, 0.11 to 0.94; $k = 8$; $I^2 = 71\%$; Figure 4). NT/TT was lower (g -0.54, -1.03 to -0.05; $k = 7$; $I^2 = 73\%$). These are small to moderate shifts toward disulfide formation. Still, the prediction intervals crossed zero for all three ratios, so a new study could well find no difference.

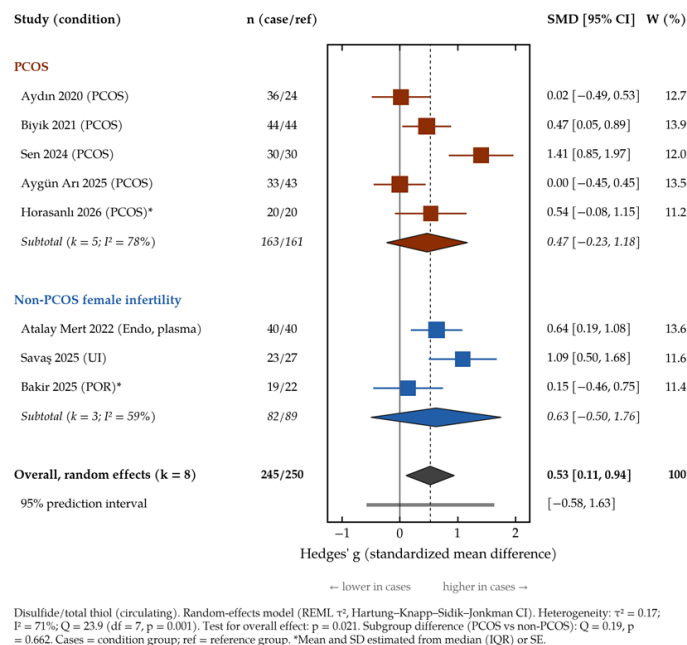


Figure 04: Forest plot of the disulfide/total thiol ratio in the serum or plasma of women with infertility-related conditions versus reference groups.

PCOS and non-PCOS subgroups did not differ (all $p \geq 0.60$ for subgroup differences). In PCOS, SS/NT reached g 0.34 (-0.01 to 0.70; $k = 5$) with little heterogeneity ($I^2 = 24\%$), while the absolute values were highly inconsistent. The non-PCOS group, which

combined endometriosis, unexplained infertility and low ovarian reserve, showed lower native thiol ($g -0.52$, -1.07 to 0.04 ; $k = 5$) with moderate heterogeneity. Figure 5 brings all pooled estimates together by subgroup and sample type.

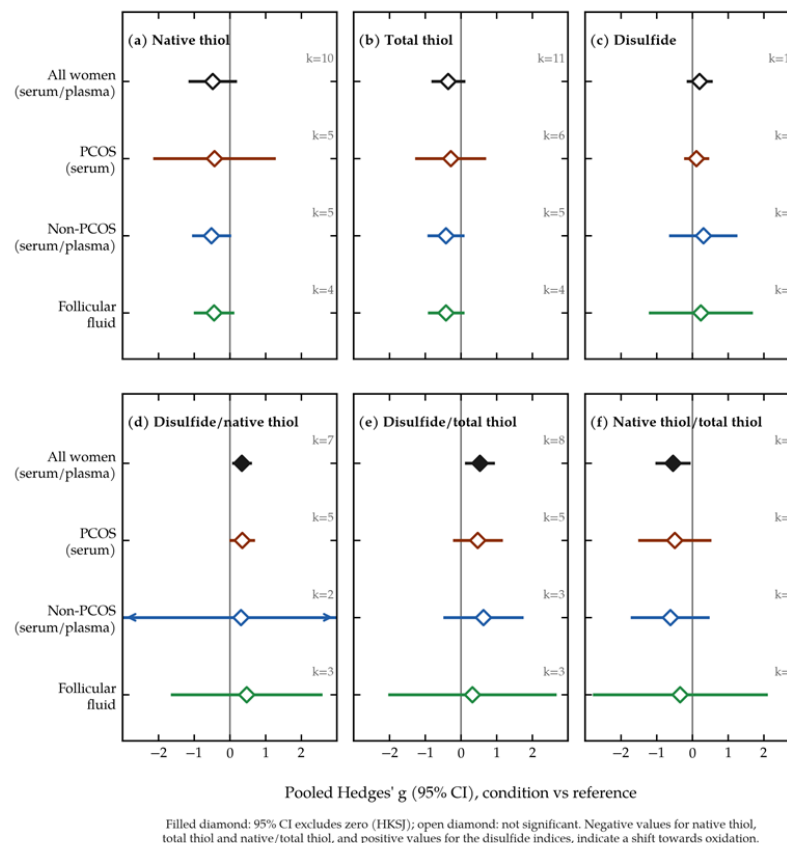


Figure 05: Pooled standardized mean differences (Hedges' g, 95% CI) for the six thiol/disulfide parameters in all women, in the PCOS and non-PCOS subgroups (serum or plasma) and in follicular fluid.

Studies without extractable data mostly agreed with this pattern. Isik et al. reported higher SS, SS/NT and SS/TT and a lower NT/TT in premature ovarian failure [49]. Bozkaya et al. found lower native and total thiol in premature ovarian insufficiency, and both fell as carotid intima-media thickness rose [50]. Turkyilmaz et al. reported lower native and total thiol in endometriosis [47]. An adult PCOS abstract showed the full oxidative pattern [38], and overweight adolescents with PCOS had lower thiols than both normal-weight peers with PCOS and controls [36]. In clomiphene-resistant PCOS, disulfide was higher than in clomiphene-sensitive women after adjustment for age and BMI [41]. The clearest exception was Yildirim et al., who found higher thiol and lower disulfide in both obese and non-obese PCOS [37]. In unexplained infertility, one study saw only non-significant shifts [51], while another found large differences and an area under the ROC curve of 0.80 for the NT/TT ratio [52].

Follicular fluid and outcomes of assisted reproduction

Four studies measured TDH in follicular fluid. Pooled native thiol ($g -0.44$, -1.01 to 0.13 ; $k = 4$; $I^2 = 38\%$) and total thiol ($g -0.42$, -0.93 to 0.09) tended to be lower in the condition groups. Disulfide and the ratios were inconsistent (I^2 88% to 90%; Supplementary Figures S7 to S12). The PCOS study gave the clearest result. Tola et al. found lower native thiol and much higher disulfide in PCOS follicular fluid (g 1.56, 0.88 to 2.25), and native thiol correlated with the fertilization rate ($r = 0.53$) [39]. Studies of low ovarian reserve did not follow this line. Turkyilmaz et al. found less disulfide in the follicular fluid of poor responders, and disulfide correlated positively with anti-Müllerian hormone and with oocyte yield [55]. Erdoğan et al. reported lower follicular and serum thiols in diminished ovarian reserve, and follicular native thiol correlated with the blastocyst quality score ($r = 0.73$) [54]. In the Iraqi study, no parameter differed between poor, normal and hyper-responders [56].

Only one study tested pregnancy directly. Serum TDH on the day of oocyte pick-up did not differ between women who became pregnant and those who did not (all $|g| \leq 0.26$). Disulfide did correlate weakly with the number of metaphase II oocytes ($\rho = 0.23$) [53]. A later follicular fluid study linked antioxidant markers to clinical pregnancy, but it did not report thiol-specific results, so it could not be used [63].

Male infertility

No male study gave group means with standard deviations in an accessible form, so the five studies were synthesized by direction of effect (Figure 6). Gul et al. compared 46 infertile and 70 fertile men with varicocele with 37 fertile controls and found higher disulfide and disulfide ratios in the infertile varicocele group [57]. Sonmez et al. grouped 117 men with varicocele by semen quality. Disulfide and both disulfide ratios differed between the groups, and each 1 $\mu\text{mol/L}$ rise in disulfide went with lower sperm concentration, progressive motility and normal morphology [58]. Coban et al. sampled the dilated spermatic vein during surgery in 61 men. Native thiol, total thiol and NT/TT were lower, and the disulfide ratios higher, than in peripheral blood from the same patients [59]. After varicocelectomy, native and total thiol rose in 56 men, and the rise correlated with changes in semen volume, motility and morphology (ρ 0.28 to 0.39) [60]. In idiopathic male infertility (79 cases, 90 controls), native and total thiol were lower and the disulfide ratios higher. Native thiol was the only independent predictor, and a cut-off of

507 $\mu\text{mol/L}$ gave 72% sensitivity and 74% specificity [61]. All 14 significant male results with a stated direction pointed toward oxidation.

Direction of effect, sensitivity analyses and small-study effects

Across the 27 case-reference comparisons, the direction of effect favored oxidation for native thiol in 18 of 22 studies (sign test $p = 0.004$) and for total thiol in 17 of 21 ($p = 0.007$). The ratios were even more one-sided: SS/NT in 14 of 15 ($p = 0.001$), SS/TT in 14 of 15 ($p = 0.001$) and NT/TT in 12 of 13 ($p = 0.003$). Disulfide itself was less clear, with 13 of 20 studies pointing toward oxidation ($p = 0.26$). Nineteen studies reported at least one significant directional difference, and 17 of them did so only in the oxidative direction (Figure 06).

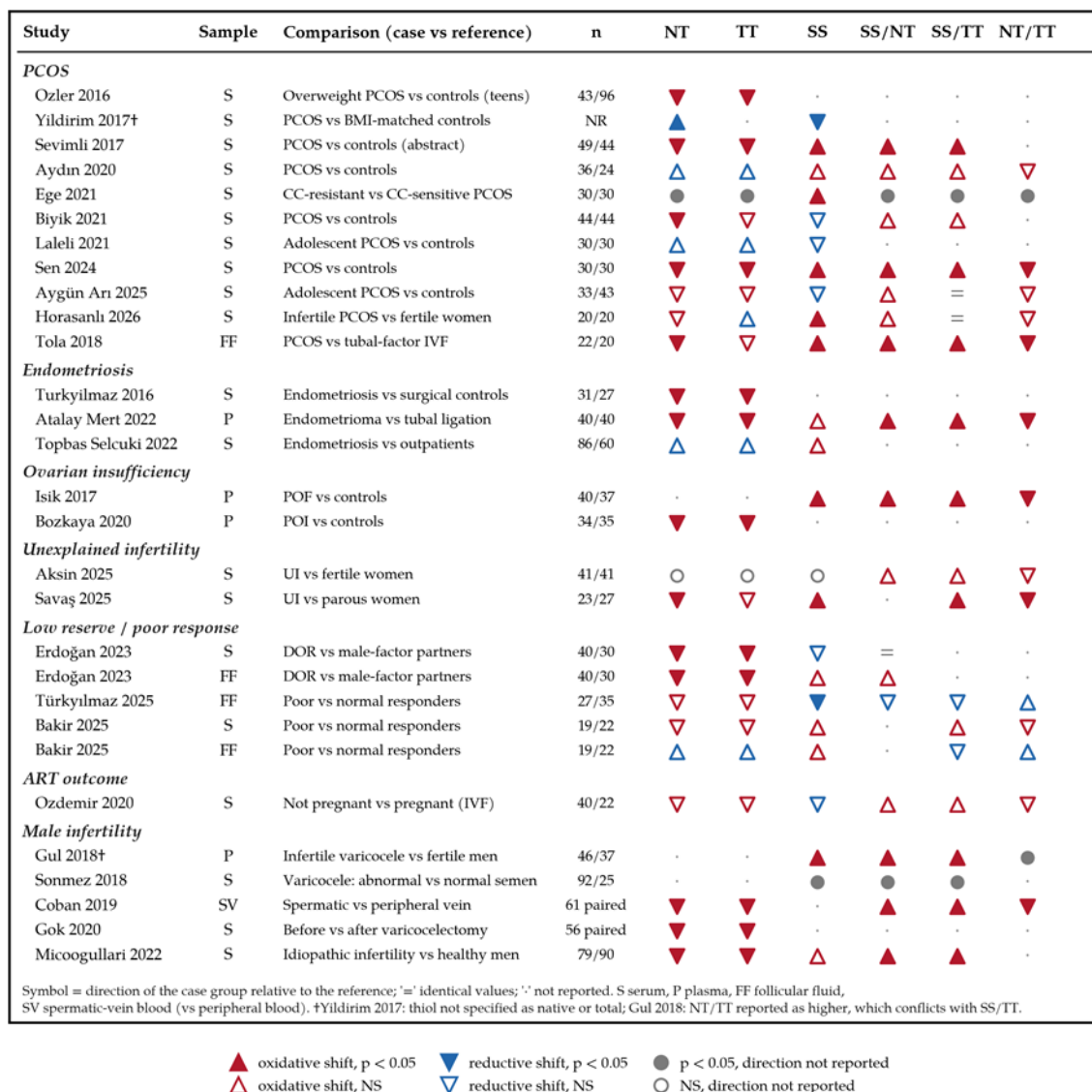


Figure 06: Effect direction plot for the 27 studies with a case-reference comparison, including those available only as abstracts. Two studies contribute both a serum and a follicular fluid row; the sign tests used one comparison per study (serum where both were available). NT, native thiol; TT, total thiol; SS, disulfide.

The pooled ratio results were fragile, though. They stayed significant with the DerSimonian-Laird estimator. In leave-one-out runs SS/TT kept its significance every time, whereas SS/NT and NT/TT lost it when certain single studies were omitted. Removing the three studies with unusual absolute values pulled all three ratios toward zero and lowered heterogeneity [21,44,52]. Two of them reported disulfide above 100 $\mu\text{mol/L}$, and one reported native thiol below 90 $\mu\text{mol/L}$. Without them, SS/NT was g 0.24 (−0.14 to 0.62), SS/TT g 0.27 (−0.14 to 0.67) and NT/TT g −0.27 (−0.64 to 0.10). The six circulating studies with a quality score of 5 or more gave the same direction without significance (SS/NT g 0.21, −0.10 to 0.52). Adding back the native thiol data of Biyik et al. in three different

ways did not change the result (g −0.44 to −0.48). Egger's test showed no clear small-study effect for native thiol ($p = 0.13$), total thiol ($p = 0.32$) or disulfide ($p = 0.08$). With ten or eleven studies, however, the test has little power (Supplementary Figures S13 to S15).

The certainty of evidence was very low for every outcome (Table 3). All data were observational, confounding was poorly controlled, heterogeneity was large for most outcomes, and the confidence intervals were wide.

DISCUSSION

Ratios rather than concentrations

As far as we know, this is the first review to gather the studies that used the automated TDH assay in male and female infertility. The picture is fairly consistent in direction but weak in size. In women, the disulfide-based ratios moved toward oxidation, while absolute thiol and disulfide concentrations were too scattered to give a clear pooled result. In men, the few available studies all pointed the same way, but none could be pooled.

Why should the ratios be any better than the concentrations? The absolute thiol levels are highly dependent on albumin, as Cys34 is the majority of the thiol pool in plasma [12,13]. NT and TT can be changed by albumin level, hydration, calibration and the analyser platform without a genuine change in redox state. This noise is damped by ratios as a decreased albumin decreases native and total thiol combined. This notion is reinforced by the diffusion of values throughout the research included. Mean native thiol varied between less than 50 and more than 600 $\mu\text{mol/L}$ and mean disulphide between approximately 12 and more than 140 $\mu\text{mol/L}$. Biology alone is unlikely to explain differences of this size. They more likely reflect reagents, calibration and sample handling. A meta-analysis in preeclampsia also found lower native and total thiol with unchanged disulfide [64], and an earlier meta-analysis in PCOS, based on other markers, concluded that circulating oxidative stress markers are abnormal whatever the body weight [65].

Biological plausibility

Several mechanisms could explain an oxidative shift in women. PCOS combines insulin resistance, androgen excess and low-grade inflammation, and each of these can raise ROS production [65,66]. Within the included studies, thiols fell as BMI, waist size and C-reactive protein rose [42,45], and obesity may explain part of the disagreement between PCOS studies [36,43]. In endometriosis, iron and heme released from retrograde menstruation drive local oxidant production [67]. The picture in low ovarian reserve is less clear. One study even found less follicular disulfide in poor responders [55]. In that study poor responders received a different gonadotropin at higher doses, and a stronger response means more follicular growth and steroid output, so the finding may reflect the stimulation more than the ovary. The same shift toward lower native and total thiol has been described in idiopathic recurrent pregnancy loss [68], which lay outside our scope.

In men, the varicocele data are the most coherent. Venous stasis, heat and hypoxia raise testicular ROS [69]. Blood from the dilated spermatic vein was more oxidized than peripheral blood of the same men [59], and thiols rose after surgical repair [60]. Taken together, this suggests a local and at least partly reversible effect. Seminal plasma deserves special attention, because spermatozoa rely on it for antioxidant protection, yet none of the included studies measured TDH in semen with the Erel method. Studies using other thiol assays found that seminal free thiols tracked sperm concentration and motility [70], and that asthenospermic men had more oxidized seminal thiols than fertile men [71]. Such cases fit the proposed category of male oxidative stress infertility [72]. Disulfides are not harmful by default, though. The protamine disulfide network is what gives the sperm nucleus its compact and protected form (Figure 7) [15,16].

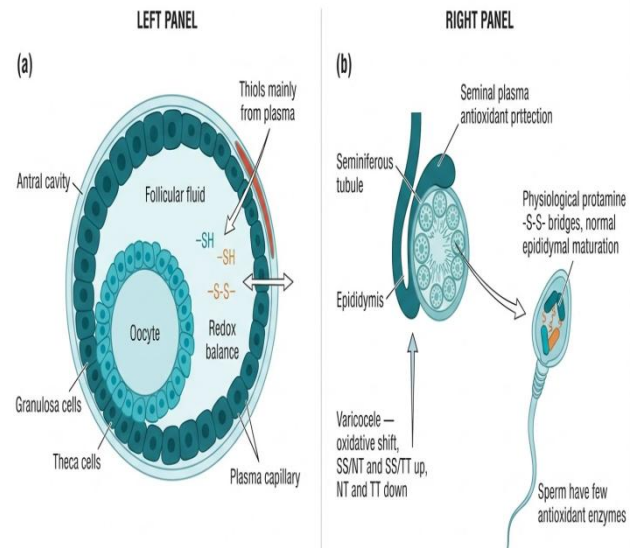


Figure 07: Proposed links between the thiol/disulfide balance and reproduction. (a) The ovarian follicle, where follicular fluid thiols come mainly from plasma. (b) The testis and the spermatozoon, with the varicocele pathway and the physiological protamine disulfide bridges of the sperm nucleus.

Clinical and analytical considerations

Would TDH be helpful in clinic? Not for now, probably. Effects were minor, prediction intervals crossed zero and the only trial that examined pregnancy revealed no change [53]. The follicular connections with fertilisation and blastocyst quality are fascinating, however they were small samples not controlled for age or ovarian reserve [39,54]. The recommended diagnostic cut-offs by two investigations have not been validated in independent samples. The test also requires a uniform standard for comparison of findings (Table 4). Thiols continue to oxidise after sample, hence the status of fasting, cycle day, time to centrifugation, storage temperature, and freeze-thaw history should be documented [19]. Measure albumin in parallel, as it drives native thiol. Follicular fluid should be clear of blood and collected from a follicle of defined size. Semen should be collected after a normal period of abstinence and processed rapidly. Quality of reporting was an issue in its own right. Two of the included studies incorrectly labelled their ratio indices [22,53] and one published an implausible standard deviation [21]. A simple internal check, that $\text{NT/TT} + 2 \text{ SS/TT} = 100\%$, would catch many similar problems.

Table 04: Suggested pre-analytical and reporting standards for thiol/disulfide studie in reproductive medicine.

Item	Why it matters	Suggested practice
Blood sampling	Thiol levels change with fasting state, time of day, cycle phase and ovarian stimulation	Fasting morning sample; record cycle day or stimulation day; in ART, sample on a fixed day such as oocyte pick-up

Item	Why it matters	Suggested practice
Processing and storage	Thiols keep oxidizing ex vivo; storage above -80°C and freeze-thaw cycles lower NT and TT and raise SS [19]	Centrifuge within one hour; store aliquots at -80°C ; avoid repeated freeze-thaw; report storage time
Follicular fluid	Blood and flushing medium change protein and thiol content	Use blood-free fluid from the first aspirate of a follicle of defined size; record follicle diameter
Semen	Abstinence time, leukocytes and delay before processing affect the redox state	Standard abstinence (2 to 7 days); process within one hour; report leukocytes and semen parameters
Albumin	Albumin Cys34 carries most plasma thiol, so NT follows albumin [12]	Measure albumin in the same sample; report albumin-adjusted or ratio indices
Confounders	BMI, age, smoking, supplements and inflammation move TDH values	Match or adjust for age and BMI; exclude or record smoking and antioxidant use; report CRP
Reporting	The three ratios are linked, and printing errors were common	Report NT, TT, SS and all three ratios with units and SD; check that $\text{NT/TT} + 2(\text{SS/TT}) = 100\%$
Laboratory practice	Batch effects and observer bias can create group differences	Blind laboratory staff to group; run cases and controls in the same batch; report assay CVs

NT, native thiol; TT, total thiol; SS, disulfide; CV, coefficient of variation; ART, assisted reproductive technology.

STRENGTHS AND LIMITATIONS

The review included both sexes and ART setting, twofold extraction for each quantitative source and small sample approaches for pooling. The direction-of-effect synthesis also helped put the abstract-only investigations into perspective. But the constraints are real. We could not search PubMed or Europe PMC directly and certain Turkish theses and conference abstracts could be overlooked, also the protocol was not recorded. Half of the studies could only be read as abstracts, among them every male study. Almost all data come from one country and from a small network of groups that includes the assay's developers, which limits generalizability and may spread shared methodological habits. Converted medians and standard errors add uncertainty. Finally, many reference groups were not of proven fertility, and some consisted of infertile women with other diagnoses, which would bias the results toward the null.

FUTURE RESEARCH

Adequately powered prospective studies should measure TDH in serum and in the local fluid (follicular fluid or seminal plasma) at the same time and follow couples to live birth. Laboratories should agree on pre-analytical rules and publish reference intervals by age, sex and BMI. Intervention studies, for example of antioxidant treatment or varicocele repair, could then test whether a change in TDH predicts a change in fertility.

CONCLUSION

Studies that used the automated Erel-Neşelioğlu assay point, on the whole, to a mild shift of the thiol/disulfide balance toward oxidation in infertile women and men. The shift shows up most clearly in the disulfide-based ratios, not in the absolute thiol or disulfide concentrations, which vary widely between laboratories. In women, pooled SS/NT and SS/TT were higher and NT/TT lower than in reference groups, but these results depended on a few studies and the certainty of evidence was very low. Follicular fluid findings were inconsistent, and a single IVF study found no link with pregnancy. The male studies, mainly on varicocele, agreed in direction but could not be pooled. TDH is cheap and easy to automate, so it deserves further study. Before it can guide decisions in infertility care or assisted reproduction, researchers need standard sample handling, routine reporting of the ratios and of albumin, independent validation of cut-offs, and larger multicenter cohorts that follow couples to live birth.

FUNDING

Nil

INFORMED CONSENT AND ETHICAL STATEMENT

Not Applicable

ACKNOWLEDGEMENT

Not Declared

REFERENCES

- Cox CM, Thoma ME, Tchangalova N, Mburu G, Bornstein MJ, Johnson CL, et al. Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis. *Hum Reprod Open*. 2022;2022(4):hoac051. doi:10.1093/hropen/hoac051
- Carson SA, Kallen AN. Diagnosis and management of infertility. *JAMA*. 2021;326(1):65-76. doi:10.1001/jama.2021.4788
- Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. *Lancet*. 2021;397(10271):319-333. doi:10.1016/S0140-6736(20)32667-2
- Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol*. 2012;10:49. doi:10.1186/1477-7827-10-49
- Aitken RJ, Drevet JR. The importance of oxidative stress in determining the functionality of mammalian spermatozoa: a two-edged sword. *Antioxidants (Basel)*. 2020;9(2):111. doi:10.3390/antiox9020111

6. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal.* 2006;8(9-10):1865-1879. doi:10.1089/ars.2006.8.1865
7. Sies H, Berndt C, Jones DP. Oxidative stress. *Annu Rev Biochem.* 2017;86:715-748. doi:10.1146/annurev-biochem-061516-045037
8. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol.* 2020;21(7):363-383. doi:10.1038/s41580-020-0230-3
9. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radic Biol Med.* 2009;47(10):1329-1338. doi:10.1016/j.freeradbiomed.2009.08.021
10. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem.* 2014;47(18):326-332. doi:10.1016/j.clinbiochem.2014.09.026
11. Erel Ö, Erdoğan S. Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical aspects. *Turk J Med Sci.* 2020;50(7):1728-1738. doi:10.3906/sag-2003-64
12. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Radic Biol Med.* 2013;65:244-253. doi:10.1016/j.freeradbiomed.2013.05.050
13. Oettl K, Marsche G. Redox state of human serum albumin in terms of cysteine-34 in health and disease. *Methods Enzymol.* 2010;474:181-195. doi:10.1016/S0076-6879(10)74011-8
14. Da Broi MG, Giorgi VSI, Wang F, Keefe DL, Albertini D, Navarro PA. Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications. *J Assist Reprod Genet.* 2018;35(5):735-751. doi:10.1007/s10815-018-1143-3
15. Balhorn R. The protamine family of sperm nuclear proteins. *Genome Biol.* 2007;8(9):227. doi:10.1186/gb-2007-8-9-227
16. Seligman J, Kosower NS, Weissenberg R, Shalgi R. Thiol-disulfide status of human sperm proteins. *J Reprod Fertil.* 1994;101(2):435-443. doi:10.1530/jrf.0.1010435
17. Kosower NS, Katayose H, Yanagimachi R. Thiol-disulfide status and acridine orange fluorescence of mammalian sperm nuclei. *J Androl.* 1992;13(4):342-348. doi:10.1002/j.1939-4640.1992.tb00335.x
18. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959;82(1):70-77. doi:10.1016/0003-9861(59)90090-6
19. Balık AR, Özbek İptec B, Yılmaz G, Kozacı LD, Erel Ö, Biçer C. Effects of storage conditions on thiol disulfide homeostasis. *Medicine Science.* 2021;10(2):450-454. doi:10.5455/medscience.2020.11.236
20. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 2021;372:n71. doi:10.1136/bmj.n71
21. Biyik I, Erten O, Isiklar OO, Ince O, Soysal C, Berikten D, et al. Comparison of serum human Klotho levels and thiol/disulfide homeostasis in women with polycystic ovary syndrome and in healthy women. *Taiwan J Obstet Gynecol.* 2021;60(3):487-491. doi:10.1016/j.tjog.2021.03.017
22. Atalay Mert S, Dilbaz B, Kinay T, Dilbaz S, Kayikcioglu F, Neselioglu S, et al. Evaluation of oxidative stress with "dynamic thiol/disulfide homeostasis" in cases with endometrioma. *Med Sci Discov.* 2022;9(8):458-464. doi:10.36472/msd.v9i8.784
23. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* 2010;25(9):603-605. doi:10.1007/s10654-010-9491-z
24. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ.* 2008;336(7650):924-926. doi:10.1136/bmj.39489.470347.AD
25. Hedges LV. Distribution theory for Glass's estimator of effect size and related estimators. *J Educ Stat.* 1981;6(2):107-128. doi:10.3102/10769986006002107
26. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res.* 2018;27(6):1785-1805. doi:10.1177/0962280216669183
27. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* 2014;14:135. doi:10.1186/1471-2288-14-135
28. IntHout J, Ioannidis JPA, Borm GF. The Hartung-Knapp-Sidik-Jonkman method for random effects meta-analysis is straightforward and considerably outperforms the standard DerSimonian-Laird method. *BMC Med Res Methodol.* 2014;14:25. doi:10.1186/1471-2288-14-25
29. Röver C, Knapp G, Friede T. Hartung-Knapp-Sidik-Jonkman approach and its modification for random-effects meta-analysis with few studies. *BMC Med Res Methodol.* 2015;15:99. doi:10.1186/s12874-015-0091-1
30. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327(7414):557-560. doi:10.1136/bmj.327.7414.557
31. IntHout J, Ioannidis JPA, Rovers MM, Goeman JJ. Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open.* 2016;6(7):e010247. doi:10.1136/bmjopen-2015-010247
32. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177-188. doi:10.1016/0197-2456(86)90046-2
33. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629-634. doi:10.1136/bmj.315.7109.629
34. Campbell M, McKenzie JE, Sowden A, Katikireddi SV, Brennan SE, Ellis S, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. *BMJ.* 2020;368:l6890. doi:10.1136/bmj.l6890
35. Thomson HJ, Thomas S. The effect direction plot: visual display of non-standardised effects across multiple outcome domains. *Res Synth Methods.* 2013;4(1):95-101. doi:10.1002/jrsm.1060
36. Ozler S, Oztas E, Tokmak A, Ergin M, Isci E, Eren F, et al. The association of thiol/disulphide homeostasis and lipid accumulation index with cardiovascular risk factors in overweight adolescents with polycystic ovary

- syndrome. Clin Endocrinol (Oxf). 2016;84(4):516-523. doi:10.1111/cen.12965
37. Yildirim M, Turkyilmaz E, Neselioglu S, Alisik M, Avsar AFY. Dynamic thiol-disulphide status in polycystic ovary syndrome and its association with the pathogenesis of the disease. Gynecol Obstet Invest. 2017;82(1):54-59. doi:10.1159/000445744
38. Sevimli H, Koca C, Senyurt M, Erel O, Laloglu E, Carlioglu A. May thiol/disulfide homeostasis predict adult PCOS? Endocr Abstr. 2017;49:EP1108. doi:10.1530/endoabs.49.ep1108
39. Tola EN, Koroğlu N, Ergin M, Oral HB, Turgut A, Erel Ö. The role of follicular fluid thiol/disulphide homeostasis in polycystic ovary syndrome. Balkan Med J. 2018;35(4):306-310. doi:10.4274/balkanmedj.2017.1140
40. Aydın GA, Turan Özsoy HG, Ankaralı H, Özgen G, Neşelioğlu S. The association of dynamic thiol-disulfide homeostasis and inflammatory markers in patients with polycystic ovary syndrome. Taiwan J Obstet Gynecol. 2020;59(1):79-84. doi:10.1016/j.tjog.2019.11.012
41. Ege S, Bademkiran MH, Peker N, Özgün Z, Bağlı İ, Erdem S, et al. Increased oxidative stress is associated with thiol/disulphide homeostasis in clomiphene citrate resistant polycystic ovary syndrome. J Obstet Gynaecol. 2021;41(3):467-470. doi:10.1080/01443615.2020.1786033
42. Laleli B, Timur B. Examination of oxidative stress level in adolescents with polycystic ovary syndrome by biochemical parameters. Gümüşhane Univ J Health Sci. 2021;10(4):935-942. doi:10.37989/gumussagbil.1003117
43. Songur Dağlı S, İlanbey B. Effect of obesity on thiol/disulfide balance in patients with polycystic ovary syndrome. Ann Clin Anal Med. 2022;13(10):1088-1091. doi:10.4328/ACAM.21217
44. Sen B, Gonultas S, Albayrak C, Temur S, Acar I, Ozkan BN, et al. Evaluation of oxidative stress and inflammation in patients with polycystic ovary syndrome. Obstet Gynecol Sci. 2024;67(4):414-420. doi:10.5468/ogs.24031
45. Aygün Arı D, Pehlivan Türk Kızılkın M, Güven AG, Kanbur N, Derman O, Erel Ö, et al. Polikistik over sendromu olan ergenlerde oksidatif stres parametrelerinin değerlendirilmesi [Evaluation of oxidative stress parameters in adolescents with polycystic ovary syndrome]. J Curr Pediatr. 2025;23(1):30-38. doi:10.4274/jcp.2025.65768
46. Horasanlı JE, Avcioğlu G, Erel Ö, Neşelioğlu S, Bağcı FÖ, Erkuş MK. Oxidative stress and thiol-disulfide levels in infertile women with polycystic ovary syndrome. Ann Clin Anal Med. 2026;17(1):23-27. doi:10.4328/ACAM.22653
47. Turkyilmaz E, Yildirim M, Cendek BD, Baran P, Alisik M, Dalgaci F, et al. Evaluation of oxidative stress markers and intra-extracellular antioxidant activities in patients with endometriosis. Eur J Obstet Gynecol Reprod Biol. 2016;199:164-168. doi:10.1016/j.ejogrb.2016.02.027
48. Topbas Selcuki NF, Yalcin Bahat P, Kaya C, Neselioglu S, Bagci K, Goksu M, et al. The role of dynamic thiol/disulfide homeostasis for the evaluation of oxidative stress in endometriosis patients. Marmara Med J. 2022;35(2):143-147. doi:10.5472/marumj.1120611
49. Isik H, Sahbaz A, Timur H, Aynioglu O, Atalay Mert S, Balık AR, et al. The use of thiol/disulfide as a novel marker in premature ovarian failure. Gynecol Obstet Invest. 2017;82(2):113-118. doi:10.1159/000445745
50. Bozkaya VÖ, Yumusak OH, Ozaksit G, Tenekcioğlu E, Gül İbrişim E, Alkan M, et al. The role of oxidative stress on subclinical atherosclerosis in premature ovarian insufficiency and relationship with carotid intima-media thickness. Gynecol Endocrinol. 2020;36(8):687-692. doi:10.1080/09513590.2020.1766439
51. Aksin Ş, Yılmaz M. The role of thiol/disulfide homeostasis in unexplained primary infertile patients: a prospective cohort study. Medicine (Baltimore). 2025;104(9):e41641. doi:10.1097/MD.00000000000041641
52. Savaş HB, Cimşir MT, Güney T. Clinical significance of thiol-disulfide homeostasis in unexplained infertility. Turk J Clin Biochem. 2025;23(2):66-78. doi:10.56615/tkdb.2025.08
53. Ozdemir AZ, Karlı P, Neşelioğlu S. Does thiol/disulfide homeostasis affect the number of metaphase 2 oocyte in the treatment of in vitro fertilization? Future Sci OA. 2020;6(2):FSO452. doi:10.2144/foa-2019-0132
54. Erdoğan K, Şanlıer N, Utlu E, Güvey H, Kahyaoğlu İ, Neşelioğlu S, et al. Serum and follicular fluid thiol/disulfide homeostasis in diminished ovarian reserve patients undergoing in vitro fertilization/intracytoplasmic sperm injection treatment. Cureus. 2023;15(2):e35476. doi:10.7759/cureus.35476
55. Turkyilmaz E, Erbaba B, Neşelioğlu S, Yılmaz NK, Tekin ÖM. Evaluation of the follicular fluid thiol/disulfide balance among patients with poor ovarian response. Clin Exp Reprod Med. 2025;52(2):134-140. doi:10.5653/cerm.2024.06863
56. Bakir NA, Al-Ghazali BS, Shakir N, Abbood MS. Association of serum and follicular fluid thiol/disulfide homeostasis in patients undergoing intracytoplasmic sperm injection. Int J Clin Obstet Gynaecol. 2025;9(1):156-163. doi:10.33545/gynae.2025.v9.i1.c.1578
57. Gul M, Bugday MS, Erel O. Thiol-disulphide homeostasis as an oxidative stress marker in men with varicocele. Andrologia. 2018;50(4):e12982. doi:10.1111/and.12982
58. Sonmez MG, Kozanhan B, Deniz CD, Iyisoy MS, Kilinc MT, Ecer G, et al. The effect of oxidative stress which can be demonstrated with thiol/disulfide homeostasis in varicocele patients on sperm parameters. Clin Lab. 2018;64(11):1823-1828. doi:10.7754/Clin.Lab.2018.180306
59. Coban S, Ustundag Y, Turkoglu AR, Guzelsoy M, Aydos MM, Keles I, et al. Thiol-disulphide balance in infertility secondary to varicocele. Andrologia. 2019;51(8):e13300. doi:10.1111/and.13300
60. Gok B, Gok G, Koc E, Hamidi N, Alijla A, Asil E, et al. The change in thiol-disulphide homeostasis levels as an oxidative stress marker after varicocelectomy: is there a relationship with sperm parameters? Andrologia. 2020;52(3):e13515. doi:10.1111/and.13515
61. Micoogullari U, Cakici MC, Kilic FU, Kisa E, Ozcift B, Caglayan A, et al. Evaluation of the role of thiol/disulfide homeostasis in the etiology of idiopathic male infertility with a novel and automated assay. Syst

- Biol Reprod Med. 2022;68(2):162-168. doi:10.1080/19396368.2021.2003481
62. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41-47. doi:10.1093/humrep/deh098
63. Korkmaz O, Karabulut S, Macit P, Cakici C. Follicular fluid oxidative stress and antioxidant defense in relation to maternal age, ovarian reserve, and in vitro fertilization outcomes. Free Radic Res. 2026;60(2):147-156. doi:10.1080/10715762.2026.2629303
64. Wang D, Yang G, You X, Zhang Z. Meta-analysis of changes in thiol-disulfide homeostasis during preeclampsia. Biomol Biomed. 2024;24(1):30-39. doi:10.17305/bb.2023.9430
65. Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update. 2013;19(3):268-288. doi:10.1093/humupd/dms059
66. Teede HJ, Tay CT, Laven J, Dokras A, Moran LJ, Piltonen TT, et al. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2023;38(9):1655-1679. doi:10.1093/humrep/dead156
67. Scutiero G, Iannone P, Bernardi G, Bonaccorsi G, Spadaro S, Volta CA, et al. Oxidative stress and endometriosis: a systematic review of the literature. Oxid Med Cell Longev. 2017;2017:7265238. doi:10.1155/2017/7265238
68. Erkenekli K, Sanhal CY, Yucel A, Bicer CK, Erel O, Uygur D. Thiol/disulfide homeostasis in patients with idiopathic recurrent pregnancy loss assessed by a novel assay: report of a preliminary study. J Obstet Gynaecol Res. 2016;42(2):136-141. doi:10.1111/jog.12860
69. Agarwal A, Hamada A, Esteves SC. Insight into oxidative stress in varicocele-associated male infertility: part I. Nat Rev Urol. 2012;9(12):678-690. doi:10.1038/nrurol.2012.197
70. Bergsma AT, Li HT, Eliveld J, Bulthuis MLC, Hoek A, van Goor H, et al. Local and systemic oxidative stress biomarkers for male infertility: the ORION study. Antioxidants (Basel). 2022;11(6):1045. doi:10.3390/antiox11061045
71. Als Salman ARS, Almashhedy LA, Hadwan MH. Effect of oral zinc supplementation on the thiol oxido-reductive index and thiol-related enzymes in seminal plasma and spermatozoa of Iraqi asthenospermic patients. Biol Trace Elem Res. 2018;184(2):340-349. doi:10.1007/s12011-017-1215-8
72. Agarwal A, Parekh N, Panner Selvam MK, Henkel R, Shah R, Homa ST, et al. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. World J Mens Health. 2019;37(3):296-312. doi:10.5534/wjmh.190055