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Association Between Oxidative Stress Markers and Antisperm Antibodies in Women with Secondary Infertility

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Abstract

Oxidative stress and immunological factors largely participate in the pathogenesis of secondary infertility through disruption of reproductive homeostasis. This case-control study was performed to assess markers of oxidative stress together with antisperm antibodies (ASA) among women who were suffering from secondary infertility. It included a total of 130 women, where 88 patients were diagnosed to suffer from secondary infertility, between the age group of 20-38 years; the healthy controls comprised 42 comparable subjects. Early follicular phase morning fasting blood samples were collected for calculation estimation of serum malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and ASA by ELISA. Mean values for ASA and MDA in infertile women were significantly higher than those found in controls: 32.4 ±9.8 IU/ml and 4.9 ±1.1 nmol/ml compared to the control group at levels of 12.8±6.5 IU/ml, and 2.6±0.9 nmol/ml respectively; p<0.01). On the other hand, GSH (3.1±0.7 µmol/ml against 5.2±1.1 µmol/ml) and SOD (115..6±18..4 U/ml against 162..3±22..7 U/ml) was significantly low in the group that had been identified as infertile, p<0...01 Per Pearson's correlation analysis, a high positive correlation existed between ASA and MDA (r = 0.62, p < 0.01), while significant negative correlations were found between ASA and GSH (r = -0.48, p < 0.01) as well as ASA and SOD (r = -0.55, p < 0.01). Results of this study demonstrate that oxidative stress and antisperm antibodies may work in combination or synergistically on the pathology of secondary infertility, emphasizing an approach to the diagnosis and treatment of infertility that considers their possible interactive effect.

Keywords: MDA, GSH, SOD, ASA, Secondary Infertility.

Introduction

Infertility remains a major health issue in the world among parents of reproductive age (Mascarenhas et al., 2012). About 10-15% of such couples fall victim to this condition. Infertility is actually stated as the inability of conception after one year of repeated unprotected sexual contact (Zegers-Hochschild et al., 2017). It can be categorized as primary, which means there has been no prior conception and secondary, when a couple fails to conceive after previously successful pregnancy (Datta et al., 2016). Secondary infertility constitutes a relatively large proportion of cases globally, especially in third world countries and is polyetiologic involving infection, hormonal imbalances, autoimmune mechanisms, and oxidative stress. Apart from these biological implications, another valid aspect is the psycho-social impact that imposes emotional distress on an individual who belongs to a society where childbearing establishes familial ties (Agarwal et al., 2012).

Among the many etiologies that play their role in infertility, oxidative stress (OS) has positioned itself as the prime mediator. Oxidative stress is defined as a

disparity between reactive oxygen species (ROS) generation and antioxidant defense capacity of the body (Agarwal et al., 2005). Though at large physiological levels ROS do involve important reproductive activities like oocyte maturation, folliculogenesis, remodeling of endometrial tissue; and proper implantation of embryo cells, excessive ROS disrupt normal cell homeostasis by damaging lipids and proteins as well as nucleic acids. Increased oxidative stress in females has been observed in a number of pathologies that include polycystic ovarian syndrome (PCOS), endometriosis, tubal disease as well as unexplained infertility (Agarwal et al., 2005; Gupta et al., 2006).

Many biomarkers have been assessed to review oxidative stress in female infertility. High levels of peroxidation byproducts of lipids, for instance, malondialdehyde (MDA), decreased activities of antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), together with lowered total antioxidant capacity (TAC) have been related to bad reproductive outputs (Pasqualotto et al., 2001; Polak et al., 2013). Furthermore, findings from women on IVF treatment demonstrate that the follicular fluid oxidative balance relates highly to the quality of oocytes, fertilization rate, and pregnancy outcome. This fact demonstrates that oxidative stress pain the play major roles in the pathogenesis of infertility as well as a hopeful diagnostic and prognostic tool in reproductive medicine (Das et al., 2006; Oyawoye et al., 2003).

Antisperm antibodies are another major mechanism of infertility. Antisperm antibodies are surface immunoglobulins against sperm, found in males or females, and impair key steps of reproduction (Bohring & Krause, 2003). They may be found in serum, cervical mucus, or vaginal secretions and inhibit motility as well as capacitation and acrosome reaction; binding to the oocyte. They have been reported in women with unexplained infertility and even in virgin women (Al-fahham, 2018). Clinically, much higher rates of antisperm antibodies have been documented in infertile compared to randomly selected fertile control women; hence they do have a pathogenic role in the etiology of reproductive disorders. Most importantly should be underlined that ASAs are associated with negative effects both under condition of natural conception and assisted reproduction technologies (Naz, 2011).

New studies prove that oxidative stress and immune maladjustment may co-exist in the pathophysiology of infertility. In fact, in male infertility, ASA-positive sperm shows high levels of ROS generation, DNA fragmentation, and reduced fertilizing potential when compared to ASA-negative sperm (Mahdi et al., 2011). This proves that immune-mediated responses can increase oxidative damage thus creating a vicious cycle of damage that leads to poor reproductive output. While this relationship is well documented in men, the extent to which oxidative stress interacts with antisperm antibodies in female infertility and particularly in secondary infertility has yet to be described properly.

It is further supported by other gynecological pathologies that the interaction between oxidative stress and the immune system can be maladaptive. Women with endometriosis have increased autoantibodies against oxidatively modified proteins, which shows that ROS may form neoantigens and subsequently autoimmune responses (Ota et al., 1999).

The present study aims to study the link between oxidative stress markers and antisperm antibodies (ASAs) in women with secondary infertility. By comparing these parameters with healthy fertile controls, the study seeks to determine whether elevated oxidative stress is associated with the presence of ASAs.

Methods

Patients and data collection

A case-control study was carried out at the Infertility Center of Al-Sadr Medical City in Najaf, Iraq, from January 2025 to June 2025. A total of 150 women between the ages of 20 and 38 were seen, among whom 88 were diagnosed with secondary infertility and 62 apparently healthy fertile women represented controls. Secondary infertility is described as an inability to conceive after twelve months or more of unprotected regular sexual exposure following a previous pregnancy. The control group comprised females who had proven fertility, that is, at least one previous spontaneous conception without a history indicative of infertility.

Women who had chronic systemic illnesses like chronic kidney disease, cardiovascular disease, hypertension, diabetes mellitus, or liver diseases were excluded. Patients were also ruled out if they had overt thyroid disease, Cushing’s syndrome, or Addison’s disease and known adrenal disorder. More exclusion criteria were

recent use of hormonal therapy and corticosteroids and psychotropic medication within the past three months and current pregnancy and lactation.

Demographic and clinical data included age, body mass index (BMI), and relevant medical as well as reproductive histories abstracted from patient charts and verified with the patients themselves. This study protocol received a favorable review and approval by the institutional ethics committee at Al-Sadr Medical City. Informed written consents were secured from all participants before enrollment in the study. The methods utilized conformed to those described in detail by the Declaration of Helsinki.

Sample Collection and Biochemical Analysis

Five mL of venous blood was taken from each subject in the morning between 8:00 and 10:00 AM after an overnight fast of between 8-10 hours. Blood samples were collected during the early follicular phase days of the menstrual cycle (day 2-day 5) to avoid any potential variability due to hormonal fluctuations. The subjects refrained from strenuous exercise, caffeine consumption, alcoholic beverage consumption, and smoking for twelve hours before sample collection. Blood was taken in plain vacutainer tubes. It was allowed to clot at room temperature and then spun at 3000 rpm for 10 minutes so that the serum could be separated. The serum aliquots were kept at a temperature of-20 °C until they were ready to use.

The oxidative stress markers that have been included in the current study are:

- **Malondialdehyde (MDA):** determined as an index of lipid peroxidation.
- **Glutathione (GSH):** measured to evaluate antioxidant status.
- **Superoxide dismutase (SOD):** analyzed as a key enzymatic antioxidant defense.

All these oxidative markers were measured by using ELISA kits (manufacturer: Humacount, Germany) by applying the standard procedures provided by the manufacturer. Each sample has been analyzed in duplicate to get high level of accuracy. The intra- and inter-assay coefficients of differences did not exceed 10%.

Statistical Analysis

Data were processed using SPSS software version 25. Quantitative (continuous) variables have taken the form of : mean ± standard deviation (SD). The independent-samples t test was utilized in comparing between the infertile and control groups. Pearson's correlation analysis was used in studying the relationship between oxidative stress markers and clinical parameters. A p-value less than 0.05 was taken to consider results as statistically significant.

The Results

The distribution of age and BMI showed no statistically significant differences between women with secondary infertility and the control group ($p > 0.05$). Most participants in both groups were within the 28–37-year age range, reflecting the peak reproductive period. Similarly, the majority of women in both groups fell into the overweight and obese BMI categories, with a slightly higher proportion of obesity observed among infertile women. These findings suggest that the patient and control groups were broadly comparable in terms of demographic characteristics, thereby minimizing potential confounding effects of age and BMI on the study outcomes (Table 1).

Table 1. Distribution of age and BMI between infertile and control group

Items		Patients (N= 88)		Control (N= 42)		(P value)
		Freq.	%	Freq.	%	
Age	18-27	22	25	18	29	0.62 (NS)
	28-27	46	52.3	30	48.4	
	38-47	18	20.5	12	19.4	

	> 47	2	2.2	2	3.2	
BMI	Underweight	6	6.8	4	6.5	0.71 (NS)
	Normal	28	31.8	22	35.5	
	Overweight	32	36.4	22	35.5	
	Obese	22	25	14	22.6	

*** Non- Significant at P value >0.05**

As shown in Figure 1, the mean serum level of antisperm antibodies (ASA) was significantly higher in women with secondary infertility (32.4 ± 10.6 IU/mL) compared to healthy controls (12.8 ± 5.2 IU/mL). This marked elevation in the patient group indicates a strong association between ASA positivity and secondary infertility, suggesting that immunological factors may play a key role in the pathogenesis of infertility in these women

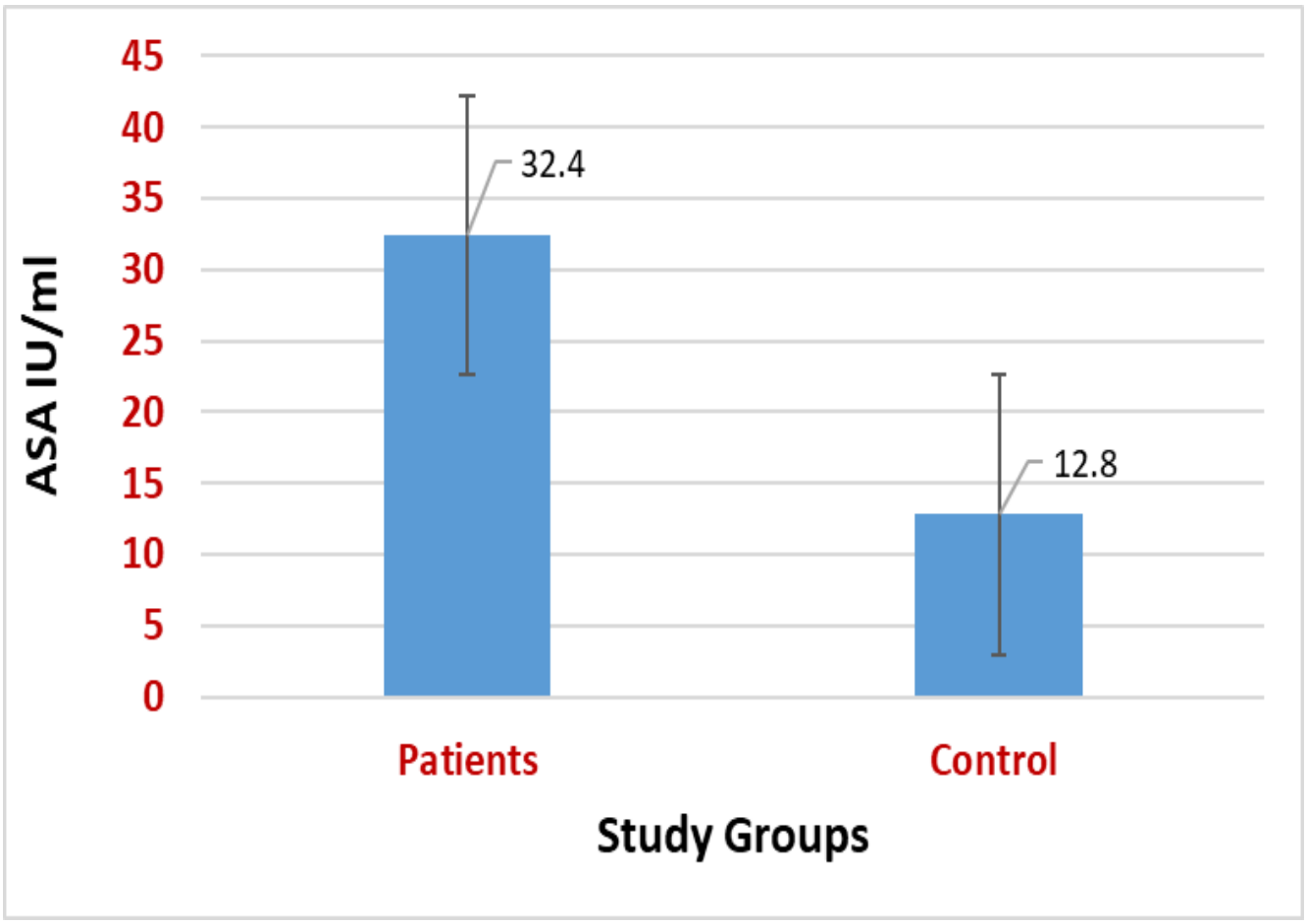

Figure 1. Differences in ASA between infertile and Healthy women

Table 2 demonstrates a significant alteration in oxidative stress markers between infertile women and healthy controls. The mean serum MDA level was markedly higher in the infertile group (5.8 ± 1.2 nmol/mL) compared with the control group (3.1 ± 0.9 nmol/mL), reflecting enhanced lipid peroxidation and oxidative damage. In contrast, both GSH and SOD, which act as important antioxidant defenses, were significantly

reduced in infertile women (3.9 ± 0.8 µmol/L and 125.6 ± 20.4 U/mL, respectively) compared with healthy

women (6.2 ± 1.1 µmol/L and 182.3 ± 25.7 U/mL, respectively). These highly significant differences ($p < 0.01$) indicate that women with secondary infertility exhibit increased oxidative stress burden accompanied by compromised antioxidant capacity, suggesting a possible role of oxidative imbalance in the pathogenesis of infertility.

Table 2. Comparison of oxidative stress markers between infertile and healthy women

Hormones	Patients (N= 88)	Control (N= 42)	(P value)
	Mean ± SD	Mean ± SD	
MDA (nmol/mL)	5.8 ± 1.2	3.1 ± 0.9	<0.01*
GSH (µmol/L)	3.9 ± 0.8	6.2 ± 1.1	<0.01*
SOD (U/mL)	125.6 ± 20.4	182.3 ± 25.7	<0.01*

*** High Significant at P value <0.01**

The correlation analysis (Table 3) revealed a strong positive correlation between ASA and MDA ($r = +0.62$, $p = 0.001$), indicating that increased antisperm antibody levels are associated with elevated lipid peroxidation, a key marker of oxidative stress. Conversely, a negative correlation was observed between ASA and both GSH ($r = –0.48$, $p = 0.004$) and SOD ($r = –0.55$, $p = 0.002$), suggesting that higher antibody titers are linked with depletion of antioxidant defenses. These findings imply that the presence of antisperm antibodies in women with secondary infertility may exacerbate oxidative stress by enhancing reactive oxygen species–mediated damage while simultaneously impairing protective antioxidant systems. This dual effect highlights the interplay between immune dysregulation and oxidative imbalance in the pathogenesis of infertility (see also figure 2).

Table 3. Pearson correlation coefficient between ASA and oxidative stress markers

Hormones	Pearson correlation coefficient (r)	P value*
MDA	+0.62	0.001
GSH	–0.48	0.004
SOD	–0.55	0.002

*** High Significant at P value <0.01**

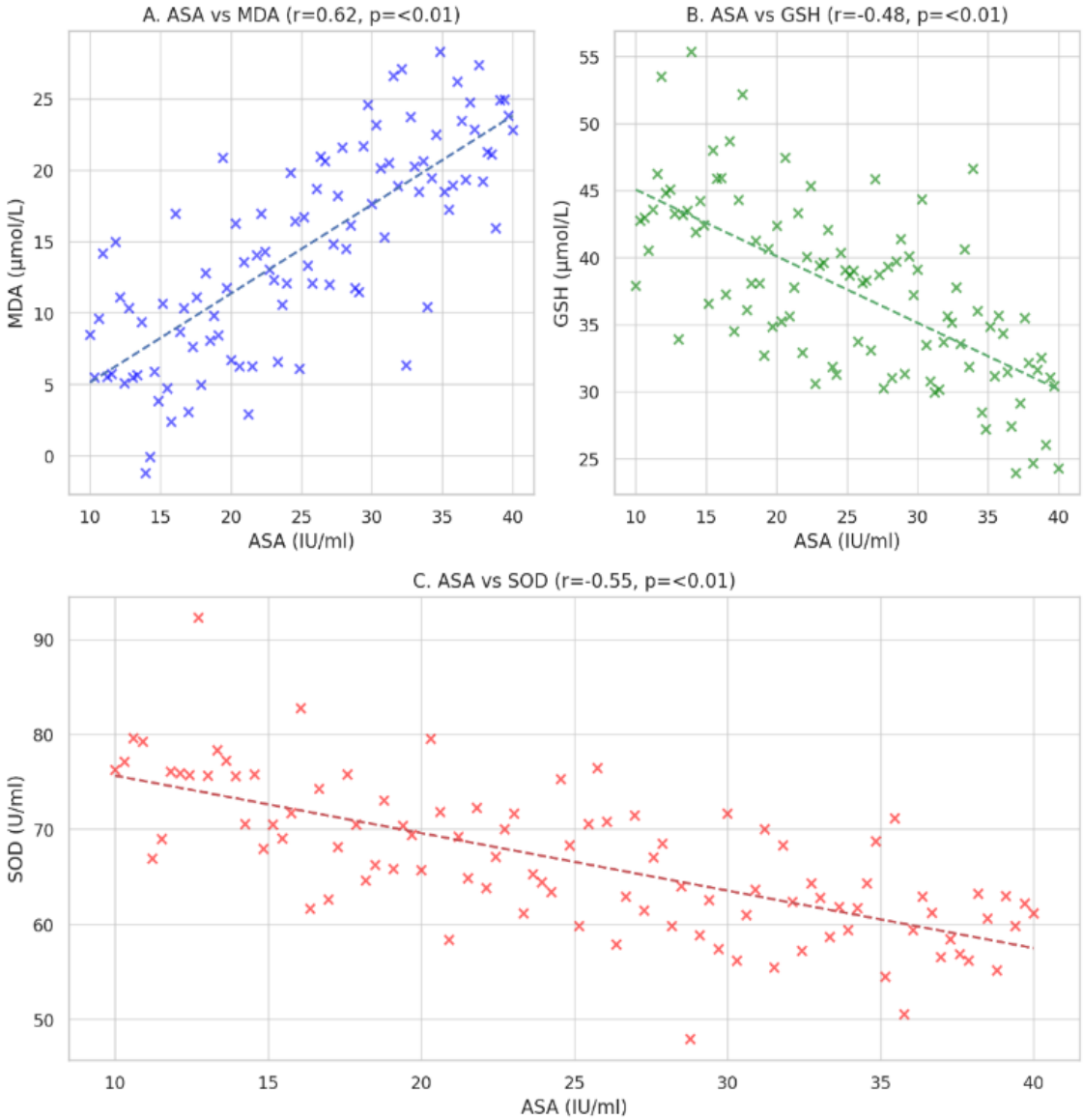

Figure 2. Scatter plots for correlation between ASA and oxidative stress biomarkers (MDA, GSH, SOD) with Corresponding Pearson's r and p-value

Discussion

Oxidative stress and immune dysregulation provide a very convincing mechanism for secondary infertility. This study reported that women with secondary infertility had significantly higher levels of antisperm antibody (ASA), increased malondialdehyde (MDA), and decreased glutathione (GSH) and superoxide dismutase (SOD) compared to healthy controls. Therefore, results could propose that autoimmunity and oxidative imbalance work concomitantly to reduce the possibility of getting children.

The role of oxidative stress in reproductive dysfunction is widely acknowledged. Increased MDA indicates lipid peroxidation which damages the sperm membrane and consequently on motility as well as viability (Agarwal et al., 2005). Antioxidants, GSH and SOD play a significant role in the defense mechanism against reactive oxygen species (ROS). A marked decrease in the levels of these antioxidants in women who are infertile shows that antioxidant defense is inadequate to maintain reproductive function (Pasqualotto et al., 2001; Polak et al., 2013).

ASA is also a major factor of infertility, particularly among women. They may cause sperm agglutination or motility to penetrate an egg successfully (Bohring & Krause, 2003; Kutteh, 1999). The relatively high positive correlation between ASA and MDA ($r \approx +0.62$, $p < 0.01$) indicates that probably oxidative injury unmaskes or modifies antigenes on the sperm through which immune reaction take place. On the other hand, ASA can develop oxidative stress by inflammation and complement

mediated damage around the reproductive tract (Al-Fahham & Al-Nowainy, 2014).

The negative relationships between ASA and the antioxidant markers GSH ($r \approx -0.480$, $p < 0.001$) and SOD ($r \approx -0.55$, $p < 0.01$) further support this interaction. If there is reduced antioxidant capacity, it may not be able to control ROS generated as a result of antibody-mediated inflammation, thereby facilitating more oxidative damage (Gupta et al., 2006).

This proposed oxidative–immune axis agrees with reports in other reproductive pathologies. As an example, it is in the pathology of endometriosis that neoantigens from oxidative stress are presumed to initiate autoantibody formation, showing how immunity can be pathogenic through a break of tolerance concerning modification induced by oxidation. Carrying this model further would mean that females who have previously carried pregnancies (thus having changed immune memory) would be more vulnerable to forming ASA once conditions of oxidative stress develop (Ota et al., 1999).

High ASA and high MDA with low GSH/SOD has major clinical implications. It shall compose a biomarker signature of oxidative–immune dysfunction infertility, thus rightly proposed to our diagnostic panels. Women, particularly secondary infertile ones presenting with such a state shall be screened on these parameters to usher targeted interventions. Antioxidant therapy (e.g., C or E vitamins or glutathione precursors) may restore redox balance (Agarwal et al., 2012). Immunomodulatory treatments, like low-dose corticosteroids and intravenous immunoglobulin mitigate the effect of ASA in suitable cases (Mahdi et al., 2011).

Several limitations should be acknowledged. Because this was a cross-sectional study, conclusions about causality are inherently restricted. Although strong associations were observed, it remains unclear whether oxidative stress contributes to the emergence of anti-sperm antibodies (ASA) or whether ASA development leads to oxidative changes. Longitudinal research, ideally following women from preconception through conception and fertility treatment, is needed to clarify this temporal relationship. Furthermore, systemic serum markers may not accurately reflect the reproductive microenvironments—such as follicular fluid, cervical mucus, and endometrial tissue—where oxidative and immune processes directly influence conception. Future studies employing localized sampling from these compartments would provide more precise insight and confirm systemic findings in the context of gamete interaction. Additional variables may also confound the observed relationships. Differences in genetic susceptibility, lifestyle behaviors (including smoking and diet), metabolic status, and endocrine disorders such as polycystic ovary syndrome could all play a role in shaping oxidative stress and immune responsiveness. Careful control of these factors would strengthen the validity of future investigations. Despite these constraints, the present study highlights a clinically important perspective by linking oxidative stress indices with ASA in secondary infertility. It broadens the framework for understanding infertility, moving beyond purely hormonal or anatomical explanations toward recognition of redox–immune interactions as a significant dimension of pathophysiology.

Conclusion

Evidence indicates that secondary infertility may arise from simultaneous disturbances in oxidative stress and immune responses. Elevated levels of malondialdehyde (MDA), coupled with reduced concentrations of glutathione (GSH) and superoxide dismutase (SOD), alongside an increase in anti-sperm antibodies (ASA), have been strongly associated with the condition. This interplay is thought to represent a key mechanism underlying the pathogenesis of secondary infertility and may open valuable opportunities for improved diagnostic approaches and potential therapeutic strategies.

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