

Correlation between some immune biomarkers and serum antisperm antibodies in men with secondary infertility

Abstract:

Inflammatory and immunological mechanisms may lead to secondary male infertility by disturbing reproductive immune homeostasis and inducing antisperm autoimmunity. This case-control study was conducted to examine whether serum antisperm antibodies (ASA) correlate with some inflammatory biomarkers, such as interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6), in a group of men with secondary infertility. In total, 180 study participants were enrolled (90 men with secondary infertility and 90 apparently healthy fertile men as control subjects). Blood samples from the peripheral veins of all subjects were obtained, and serums ASA, IL-1 β and IL-6 were measured enzyme-linked immunosorbent assay (ELISA). Men with secondary infertility exhibited significantly higher serum concentrations of IL-1 β (8.42 ± 2.31 pg/mL) in comparison to healthy controls (6.91 ± 1.84 pg/mL; $P < 0.02$). Likewise, serum IL-6 was significantly higher in infertile men (12.76 ± 3.48 pg/mL) than in controls (10.84 ± 2.91 pg/mL; $P < 0.03$). Pearson correlation coefficient revealed a significant positive correlation between ASA and IL-1 β ($r = +0.322$, $P=0.002$) and IL-6 ($r = +0.452$, $P=0.003$). Higher inflammatory activity, especially with IL-1 β and IL-6 may correlate with serum antisperm antibody levels in the secondary infertility male. More investigations are necessary to elucidate the mechanisms for these associations, and to explore whether these biomarkers have any clinical significance.

Keywords: IL-1 β , IL-6, ASA, Secondary Infertility,

Introduction

Infertility constitutes a significant area of reproductive health problem for individuals and couples across the world, with male factor issues making a

considerable contribution to the magnitude of the problem (Gül et al., 2024). According to current evidence, male factors are considered a primary or contributing cause in almost 50% of infertility cases, yet routine assessment of semen is still insufficient to explain impaired reproductive potential (Dhikhirullahi & Zhang, 2025). Hence, more attention has been paid to the underlying molecular and immunological mechanisms that lead to abnormal spermatogenesis, breast spermatozoa, and fertilization failure. World Health Organization (WHO) recognized that the assessment of male reproductive factors is a paramount aspect of a comprehensive approach to infertility diagnosis and management (World Health Organization [WHO], 2025). Advances in reproductive immunology itself revealed the complicated interactions of the immune system and the male sexual tracts, in which tightly regulated immune mechanisms are required for sustaining spermatogenesis while maintaining tolerance against developing germ cells (Liu et al., 2026). The male reproductive tract has evolved to have specialized immune-protective mechanisms which include the blood–testis barrier and local immunoregulatory networks to limit inappropriate immunological recognition of spermatozoal antigens (Hedger, 2010). Infection, inflammation, trauma, obstruction, surgery, or other reproductive disorders can interfere with these protective mechanisms and uncover spermatogenic antigens to the immunological system, resulting in the formation of antisperm antibodies (ASA). They are immunoglobulins against sperm surface antigens, and could influence many steps of sperm function, such as motility, capacitation, acrosomal activity, interaction with cervical mucus and sperm–oocyte recognition (Sciorio et al., 2025). While the clinical significance of ASA remain incompletely defined, the evidence continues to accumulate which support that immune factors, including ASA may play a role in exerting an immunological component to male infertility, cited by several recent studies (Silva et al., 2021 and Lindenbaum et al., 2026).

The ASA are particularly pertinent; in addition to autoantibody production, inflammatory mediators may contribute to alterations in the male reproductive microenvironment. Cytokine activity in sperm maturation, survival and function entails interaction between immune, epithelial and reproductive cells. Excess production of pro-inflammatory mediators which include, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and various other cytokines have been reported to be associated with male reproductive disorders (Haratian et al., 2025). A recently published systematic review and meta-analysis found that seminal-plasma concentrations of IL-6, TNF- α and CXCL8 were significantly greater in infertile men than controls, but IL-6 showed substantial heterogeneity (Lyons et al., 2026). Moreover, conditions arising after a previous conception, such as genital tract infection, inflammation, obstruction, trauma, or surgical intervention, may disrupt immune tolerance to spermatozoa and provide insight into the biological mechanisms underlying the development of ASA (Fijak et al., 2018). Factors explored that impacted ASA production, according to a recent systematic review, fell within the categories of infectious, inflammatory, anatomical, and surgical conditions; with vasectomy, vasectomy reversal, genital infections, varicocele, previous reproductive tract procedures all cited as associated conditions. This is especially relevant in the context of secondary infertility, as a number of these conditions may arise after a prior established reproductive function (Haratian et al., 2025).

The connection between inflammatory cytokines and ASA is of particular interest, as inflammation possibly represents one mechanism by which the male reproductive tract's immune privilege is compromised. The selection of immune-cells and production of cytokines at the site of local infection or inflammatory activity can disrupt the integrity of reproductive barriers and allow immune exposure to sperm antigens (Loveland et al., 2017). Clinical studies have previously found an association for more than one mechanism including IL-6, TNF- α , genital inflammation, leukocytospermia and ASA which

indicates that in some patients, inflammatory and autoimmune mechanisms coexist (. Nevertheless, it is still unclear which direction the relationships take, for example, whether increased cytokine concentrations mediate the ASA formation or merely represent a by-product of an underlying inflammatory disorder in infertility (Eldamnhoury et al., 2018; Martínez-Prado and Camejo Bermúdez, 2010).

Thus, the assessment of immune markers in conjunction with ASA may further help us in elucidating the immunological aspect of secondary male infertility. This approach might help to determine if systemic inflammatory activity mediates in any way the appearance or extent of sperm-directed autoimmunity and may bolster the description of patients whose infertility cannot be adequately characterized by routine semen parameters alone (Chomany et al., 2026). Emerging studies of seminal and systemic immune markers as possible adjuncts to the routine work-up of male infertility have gained attention, though these methods remain limited by broad methodological variations and a lack of threshold or normative values (Vashisht & Gahlay, 2024; Lyons et al., 2026).

Accordingly, the present study seeks to study the role of interleukin-6 (IL-6) and interleukin-1 beta (IL-1 β) and in the expected inflammatory response in male secondary infertility and their association with serum antisperm antibodies.

Methods

Patients and data collection

This study was enrolled in the case-control study design and conducted at Al-Diwaniya Teaching Hospital, in Al-Diwaniya City, Iraq from December 2025 to April 2026. This study included 180 adult men: 90 patients with a diagnosis of secondary infertility, and 90 apparently healthy fertile men as a control group. Secondary male infertility was defined as a failure to conceive with a female partner after a minimum of 12 months unprotected regular sexual intercourse in

a previously fertile couple (Hibi et al., 2022). In the control group, individuals with proven fertility (a previous child) and no history of infertility and significant reproductive disorders participated.

Exclusion criteria included men with primary infertility, known genetic or chromosomal abnormalities affecting fertility, previous bilateral orchiectomy, testicular malignancy, or severe chronic systemic diseases. Demographic and clinical characteristics were extracted from a standardized data collection form and confirmed by direct interviews and available medical records. The information addressed included age, body mass index (BMI). The study protocol received review and approval to conduct research experiments by the institutional ethics committee of Al-Diwaniya Teaching Hospital. All individuals presented written informed consent before participation. All interventions in this study were performed according to the ethical principles of the Declaration of Helsinki and where applicable with relevant institutional requirements regarding research on human participants.

Sample Collection and Biochemical Analysis

Each participant provided samples of peripheral venous blood in quantity of 5 ml during the morning after an overnight fast of approximately 8–10 hours. All blood samples were collected into sterile plain vacuum tubes and permitted to clot at room temperature. They were then subjected to centrifugation for 10 min at 3,000 rpm for serum separation. Serum has been aliquoted into sterile tubes, labeled, and stored at -20°C until analysis. Avoided multiple freeze thaw cycle – to limit any degradation of analyzed biomarkers.

Determination of Serum ASA and Immune Biomarkers

The major immune parameters measured in the current study were serum ASA, IL- 1β , and IL-6. Human Anti-AsAb (Anti-Sperm Antibody) enzyme-linked immunosorbent assay (ELISA) Kit (ELK Biotechnology, China; Cat.) was

bound to resolve ASA concentrations in serum. No. ELK8071), which is a sandwich enzyme immunoassay for the quantitative determination of antisperm antibodies in human serum. Serum concentrations of IL-1 β were evaluated using Human IL-1 beta ELISA Kit (OriGene Technologies, USA; Cat. No. EA100263), while serum IL-6 concentrations were measured utilizing Human IL-6 ELISA Kit (OriGene Technologies, USA; Cat. No. EA100279). Both assays are additional quantitative sandwich ELISA protocols validated for the determination of analytes in human serum and plasma. All assays were conducted per the directions of the manufacturers. In each assay run, standards, blank samples and quality controls were included. All of the serum samples were analyzed in duplicates, and we used the mean value of the duplicate measurements in further statistical analysis. Levels of IL-1 β and IL-6 in serum were reported in pg/mL, but concentrations of ASA were reported depending on the unit provided by the calibration system of each assay.

Statistical Analysis

Data were calculated utilizing SPSS software version 25. Quantitative (continuous) variables have taken the form of mean $\pm$ 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 Age and BMI distributions of men with secondary infertility and the healthy control group are presented in table 1. The majority of participants were aged between 35–44 for both men and women in both groups. There was no statistically significant difference in the age ratio of patients to controls ($P = 0.56$). Worthy of note, with respect to BMI, was that the largest proportion of

the infertile population were overweight (40.0%) but the largest proportion of the control population was normal-weight (42.2%). A slightly higher percentage of the infertile subjects were overweight and obese; however, the BMI distribution is not significantly different between the two groups ($P = 0.09$).

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

Items		Patients (N= 90)		Control (N= 90)		(P value)
		Freq.	%	Freq.	%	
Age	24-35	28	31.1	31	34.4	0.56 (NS)
	35-44	32	35.6	30	33.3	
	45-55	22	24.4	20	22.2	
	> 55	8	8.9	9	10	
BMI	Underweight	5	5.6	7	7.8	0.09 (NS)
	Normal	30	33.3	38	42.2	
	Overweight	36	40	32	35.6	
	Obese	19	21.1	13	14.4	

* Non- Significant at P value >0.05

As shown in Figure 1, the mean serum level of antisperm antibodies (ASA) was significantly higher in men with secondary infertility (62.5 ± 22.3 IU/mL) compared to healthy controls (14.7 ± 6.3 IU/mL).

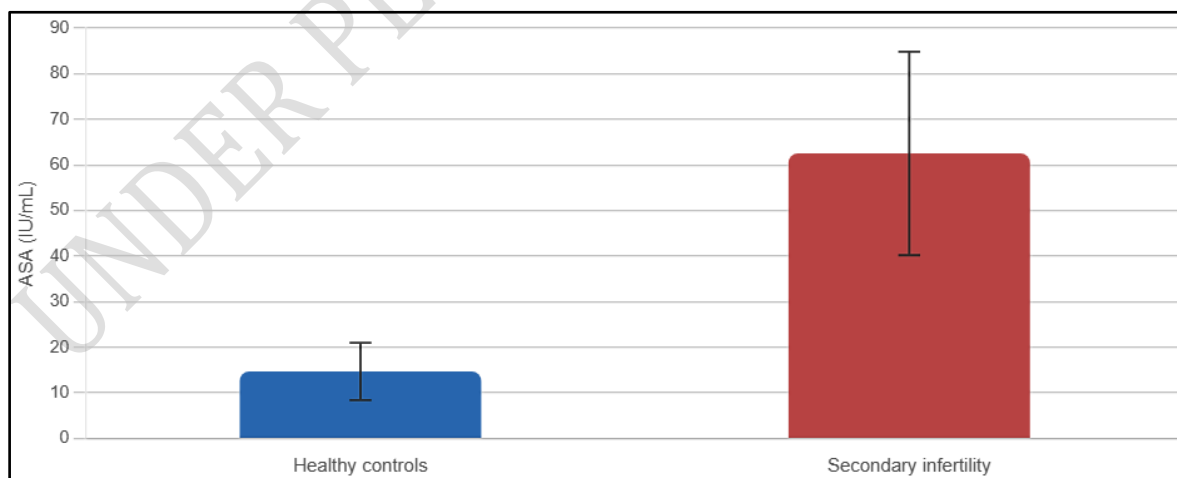


Figure 1. Differences in ASA between infertile and Healthy men

Table 2 shows serum levels of IL-1 β and IL-6 in men with secondary infertility and healthy controls. Serum levels of IL-1 β in the infertile group (8.42 ± 2.31 pg/mL) were also significantly greater than those in controls (6.91 ± 1.84 pg/mL) ($P < 0.02$). Moreover, the mean concentration of IL-6 was also significantly higher in infertile men (12.76 ± 3.48 pg/mL) than in healthy controls (10.84 ± 2.91 pg/mL) ($P < 0.03$).

Table 2. Comparison of IL-1 β and IL-6 between infertile and healthy men

Hormones	Patients (N= 90)	Control (N= 90)	(P value)
	Mean $\pm$ SD	Mean $\pm$ SD	
IL-1 β (pg/mL)	8.42 ± 2.31	6.91 ± 1.84	$<0.02^*$
IL-6 (pg/mL)	12.76 ± 3.48	10.84 ± 2.91	$<0.03^*$

* Significant at P value <0.05

The correlation testing (Table 3) revealed a strong positive correlation between ASA and each of IL-1 β ($r = +0.332$, $p = 0.002$), and IL-6 ($r = +0.452$, $p = 0.003$), indicating that increased antisperm antibody levels are associated with elevated immune markers (see also figure 2).

Table 3. Pearson correlation coefficient between ASA and immune markers

Markers	Pearson correlation coefficient (r)	P value*
IL-1 β	$+0.322$	0.002
IL-6	$+0.452$	0.003

* High Significant at P value <0.01

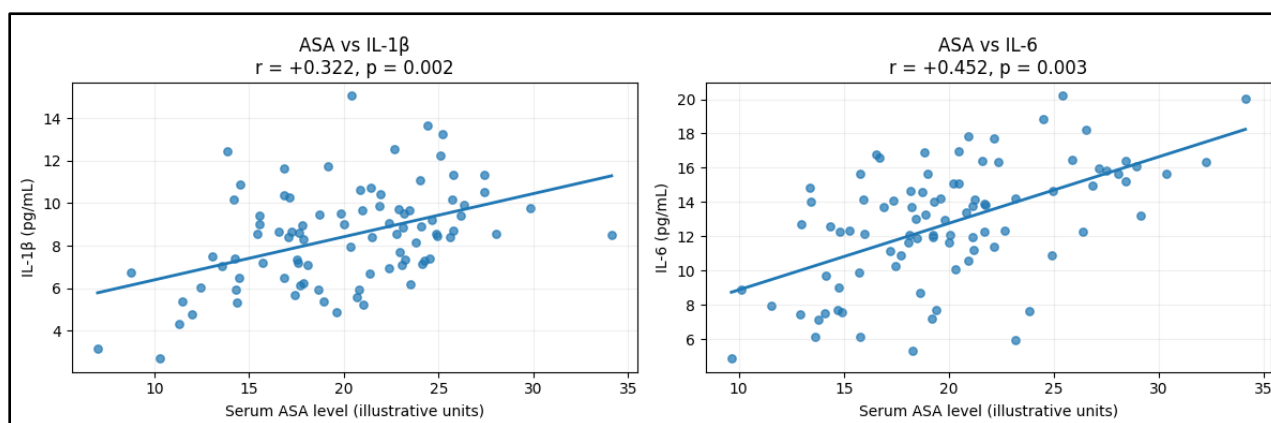


Figure 2. Scatter plots for correlation between ASA and immune markers with Corresponding Pearson's r and p -values

Discussion

The current study assessed the association of various inflammatory immune biomarkers and serum antisperm antibodies (ASAs) in men with secondary infertility. Demographic findings revealed no statistically significant differences between the infertile group and healthy control subjects with respect to age ($P=0.56$) or BMI ($P=0.09$). The relative comparability is also important as the presence of large disparities in these characteristics may confound the inferences related to inflammatory biomarkers. The fraction of overweight and obese subjects in the infertile group was slightly larger but still non-significant. As such, the observed differences in IL-1 β , IL-6, and their associations with ASA in the current sample cannot be easily explained by differences in age or BMI.

The main result was that patients' serum IL-1 β concentration in the men with secondary infertility was significantly higher than the healthy controls. IL-1 β is a potent pro-inflammatory cytokine that exerts a vital role in both the startup and progression of inflammatory responses and is synthesized in a variety of settings following the activation of innate immune pathways (Lopez-Castejon & Brough, 2011). In the male reproductive system, inflammatory mediators can alter testicular and epididymal function and can disrupt the necessary

immunological environment for normal progression of viable spermatozoa. Prior studies have yielded evidence for heightened inflammatory activities in male infertility (Hedger, 2011). For instance, a study previously reported that the cytokine interleukin 1 β (IL-1 β) could distinguish between fertile and infertile ovaries, emphasizing its potential as a marker of inflammation-related reproductive dysfunctions in the female reproductive tract (Yang et al., 2018). Also, a study of male and female cytokines could point to the relationship between inflammatory cytokines and male reproductive disease, our own results have shown that cytokine profiles differ between men of fertile and infertile male and IL-1 α and other inflammatory cytokines can be useful markers of male reproduction disease (Loveland et al., 2017).

However, the current result regarding IL-1 β should be taken cautiously. The evidence surrounding this cytokine is somewhat inconsistent. In a meta-analysis and systematic review of 52 studies, with a total of 8,153 men, a large 2026 analysis showed that IL-6 was significantly higher in the seminal plasma of men with infertility, while IL-1 β showed no significant overall variation between the two groups of men (infertile and fertile/normozoospermic). Substantial heterogeneity across definitions of infertility and study populations was emphasised by the authors (Lyons et al, 2026). The results suggest that the high serum IL-1 β was not a general characteristic of male infertility but rather reflective of the systemic inflammation present in the specific subjects studied in the present paper.

IL-6 showed a similar but more consistent pattern. Men with secondary infertility had significantly higher serum IL-6 concentrations than healthy controls ($P < 0.03$). This finding is in agreement with ample prior evidence implicating IL-6 in mediating male reproductive dysfunction (Koumba et al., 2026 ; Lyons et al., 2026). Previous studies showed seminal plasma IL-6 concentrations were also significantly higher in infertile men compared with fertile or normozoospermic controls (Płaczkowska et al., 2024). Similar findings

have been shown previously: elevated concentrations of IL-6 in seminal plasma obtained from both infertile and immunoinfertile men compared with fertile men, with significant relationships between IL-6 concentrations and selected sperm-function parameters (Naz & Kaplan, 1994). In a similar Iraqi study, serum IL-6 and ASA levels were observed to be significantly elevated in infertile men in comparison to with fertile controls, providing regional corroboration to the data presented in this study (Taqi & Al-Murshidi, 2022).

The most significant approach with the result of this study was the positive relationship between serum ASA and both inflammatory biomarkers. ASA had a statistically positive correlation with both IL-1 β ($r = +0.322$; $P = 0.002$, < 0.001) and IL-6 ($r = +0.452$; $P = 0.003$). Results provide some evidence for systematic differences between men in the circulating concentrations of ASA and inflammatory cytokines. This pattern seems biologically plausible because loss of immune tolerance to sperm may follow inflammation, tissue injury, infection or rupture of the blood–testis or blood–epididymis barriers (Lyons et al., 2023). In a recent systematic review, Haratian et al. (2025) determined several aspects associated with ASA production in infertile men, including infections, varicocele, vasectomy, and diseases able to affect the reproductive tract immune homeostasis.

Previous Iraqi research findings also provides partial support for the current correlation results. Taqi and Al-Murshidi (2022) conducted a study in Al-Najaf City and showed significant correlations between seminal concentrations of ASA, IL-1 and IL-6 with leukocyte concentrations. Their investigation determined that the presence of leukocytes correlated with greater concentrations of IL-1 and IL-6, and both ASA and these cytokines correlated with worse semen quality. This regional evidence is particularly relevant as the present study also examined an Iraqi male infertility population, albeit via different biological specimens and study designs.

287 However, the current findings are inconsistent with those by Eggert-Kruse et al.
288 (2007) focused on IL-1 β and TNF- α in seminal plasma from 148 asymptomatic
289 men from couples with subfertility. While there were significant associations
290 between seminal leukocyte measures and IL-1 β , other local IgG or IgA ASA
291 had little correlation with IL-1 β . Such a difference may be due to significant
292 differences in methodology. The current study assessed serum ASA and
293 cytokines in men with secondary infertility while Eggert-Kruse et al. Analyzed
294 seminal plasma cytokines and local ASA. Cytokine levels in serum and seminal
295 plasma may reflect different biological compartments, thus circulating
296 inflammatory activity does not always correlate well with the local reproductive
297 tract environment. In fact, recent studies highlight the potential for substantial
298 differences in cytokine concentrations and profiles in serum as compared with
299 seminal plasma (Havrylyuk et al., 2015).

300 The positive correlational findings in this current study should thus be treated as
301 relational associations instead of that causal proof. Characteristics of this
302 inflammatory environment include increased IL-1 β and IL-6, either of which
303 could promote disruption of reproductive immune tolerance leading to ASA
304 formation or alternatively conduct an immune response directed against his own
305 sperm that in turn causes such inflammatory signaling (Mazloomi et al., 2023).
306 Another option is that both phenomena are separate responses to a third
307 unmeasured outcome (eg, prior genital tract inflammation or violation of
308 reproductive immune barriers). This design does not allow for disentangling of
309 these possibilities at present, which is the case-control and correlational nature
310 of this data (Nguyen et al., 2014) .

312 **Conclusion**

313 The current study showed highly greater serum levels of IL-1 β and IL-6 in the
314 secondary infertile men compared with healthy controls. Serum antisperm
315 antibodies correlated positively with both IL-1 β and IL-6, which suggests an

association between sperm-directed autoimmunity and inflammation. The current observation of a stronger correlation of IL-6 and ASA confirms its significant involvement in the inflammatory processes that lead to secondary male infertility. Additional longitudinal and mechanistic studies are needed to accurately define the biological relationship between inflammatory cytokines and antisperm antibody production and to evaluate their clinical utility.

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