

REVIEWER'S REPORT

Manuscript No.: JNHM- 174

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

Recommendation:

Accept after minor revision

Rating	Excel.	Good	Fair	Poor
Originality			✓	
Techn. Quality			✓	
Clarity		✓		
Significance	✓			

Reviewer Name: Bilqees Hamza

Detailed Reviewer's Report

General Overview

This manuscript evaluates the relationship between systemic pro-inflammatory cytokines—specifically interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6)—and serum antisperm antibodies (ASA) in men diagnosed with secondary infertility. Conducting a case-control design at Al-Diwaniya Teaching Hospital in Iraq, the authors compared 90 men with secondary infertility against 90 apparently healthy fertile controls. Serum concentrations of ASA, IL-1 β , and IL-6 were quantified via enzyme-linked immunosorbent assay (ELISA).

The primary findings demonstrate that men with secondary infertility exhibit statistically significant elevations in serum IL-1 β (8.42 ± 2.31 pg/mL) vs. 6.91 ± 1.84 pg/mL; $P < 0.02$) and serum IL-6 (12.76 ± 3.48 pg/mL) vs. 10.84 ± 2.91 pg/mL; $P < 0.03$) compared to fertile controls. Furthermore, serum ASA concentrations were markedly higher in the infertile group (62.5 ± 22.3 IU/mL) vs. 14.7 ± 6.3 IU/mL), showing significant positive Pearson correlations with both IL-1 β ($r = +0.322$, $P = 0.002$) and IL-6 ($r = +0.452$, $P = 0.003$).

The manuscript addresses a relevant topic in reproductive immunology, highlighting how systemic low-grade inflammation may accompany or contribute to sperm-directed autoimmunity in secondary male infertility. The study design is straightforward, the sample size ($N = 180$) is adequate for primary

REVIEWER'S REPORT

correlation analysis, and the discussion appropriately contextualizes the findings within both local Iraqi and broader international literature.

Key Strengths

1. **Well-Defined Cohort Selection:** Comparing secondary infertility cases against age- and BMI-matched fertile controls helps minimize common systemic confounders that could independently elevate inflammatory markers.
2. **Clear Objective and Methodological Rationale:** Focus on secondary male infertility isolates cases where immune tolerance to sperm antigens may have been disrupted following an established prior fertility event (e.g., subsequent infection, trauma, or varicocele).
3. **Sound Biological Relevance:** Demonstrating a significant link between pro-inflammatory cytokines (IL-1 β , IL-6) and circulating antisperm antibodies provides empirical support for the hypothesis that inflammatory responses and autoimmune reactivity coexist in infertile males.
4. **Honest Discussion of Methodological Limitations:** The authors acknowledge key literature inconsistencies—such as differences between systemic (serum) and localized (seminal plasma) compartments—and correctly avoid drawing definitive causal conclusions from cross-sectional data.

Recommendations for Improvement

While the manuscript is scientifically valid and worthy of publication, a few minor revisions will enhance its clarity, methodological precision, and presentation:

1. **Clarify Discrepancy in Abstract vs. Main Text Correlation Values:**

In the abstract, the correlation between ASA and IL-1 β is listed as $r = +0.322$ ($P = 0.002$). However, in Section 5 (Discussion, line 267), the text reads " $r = +0.322$; $P = 0.002$, < 0.001 ". Ensure that P -values and correlation coefficients are reported consistently throughout the abstract, results tables, and discussion text.

2. **Reconcile X-Axis Labels in Figure Scatter Plots:**

In Figure 2, the scatter plots label the x-axis as "Serum ASA level (illustrative units)" ranging from 10 to 35, whereas Table 1 / Figure 1 report mean serum ASA levels as 62.5 ± 22.3 IU/mL for infertile men and 14.7 ± 6.3 IU/mL for controls. Please update the x-axis labels in Figure 2 to reflect the actual calibrated units (IU/mL) and data range matching the primary results.

3. **Incorporate Basic Semen Analysis Data if Available:**

The study evaluates serum ASA and systemic cytokines. If semen analysis parameters (such as sperm concentration, motility, morphology, or leukocytospermia counts) were routinely collected during patient

REVIEWER'S REPORT

enrollment, briefly summarizing or correlating these parameters with serum ASA/IL-6 would significantly strengthen the clinical context.

4. Address Departmental/Laboratory Quality Control Details:

In Section 2 (Methods), briefly state the inter-assay and intra-assay coefficients of variation ($\text{CV}\%$) for the ELISA kits used (ELK Biotechnology and OriGene) to confirm assay precision and reproducibility.

5. Minor Proofreading and Editorial Corrections:

- Fix minor typos in text: e.g., "breast spermatozoa" in line 36 (likely intended as "impaired spermatozoa"), "investigationsarenecessary" in lines 23–24, "individualspresented" in line 128, and "calculatedutilizing" in line 158.
- Ensure uniform formatting of P -value notation (e.g., standardizing $P < 0.02$ vs $p = 0.002$) across text and tables.

Final Evaluation and Verdict

This study makes a meaningful contribution to understanding the immune-inflammatory profile of men with secondary infertility. The methodology is sound, and the conclusions are well-supported by the empirical data presented.

The manuscript is **accepted with minor revisions**. Resolving the minor textual inconsistencies, unit alignments in figures, and typographical edits outlined above will render the paper fully polished for publication