**ELISA-BASED DETECTION OF IMMUNOGLOBULIN CLASSES OF ANTISPERM ANTIBODIES IN INFERTILE MALES AND FEMALES IN EDO STATE, NIGERIA**

**ABSTRACT**

Immune infertility is a significant cause of infertility in humans, associated with the production of antisperm antibodies detectable in body fluids. This study aimed to investigate the prevalence of IgA, IgG, and IgM immunoglobulin classes of antisperm antibodies in males and females, as well as the relationship between these antibodies and sex variation in their incidence, in Edo State, Nigeria. The study population consisted of 96 participants who tested positive for antisperm antibodies using the Slide Latex Agglutination Test, comprising 28 males and 68 females. The immunoglobulin classes of antisperm antibodies were identified using the Antispermatozoa Antibody ELISA (Enzyme-Linked Immunosorbent Assay) Ig-Classifying test. Statistical analysis using the one-way chi-square test revealed that males had a significantly lower incidence of IgA (27.8%, p < .01) and IgM (26.1%, p < .05) compared to females (72.2% and 73.9%, respectively). In contrast, no significant difference was observed in IgG between males and females (p = .251). These findings suggest that IgA is the most prevalent class of antisperm antibodies, with a higher incidence in females than males. Understanding the distribution of antisperm antibody classes can help inform targeted diagnostic approaches and fertility counseling. In resource-limited settings, prioritizing ELISA-based screening for specific immunoglobulin classes may enhance early detection and guide cost-effective treatment strategies.

**Keywords: Antisperm Antibodies, Infertility, Immunoglobulins, Prevalence, ELISA**

**1.0 Introduction**

Infertility remains a pressing global health issue, affecting an estimated 10 - 15% of couples of reproductive age worldwide (Mascarenhas *et al.,* 2012). It is a condition with multifactorial etiology, involving anatomical, hormonal, genetic, infectious, and increasingly recognized immunological causes. Among these, immune infertility particularly the presence of antisperm antibodies (ASAs) is a growing area of interest due to its significant, yet often underdiagnosed, role in unexplained infertility cases (Clarke *et al.,* 2006; Francavilla *et al.,* 2007).

ASAs are autoantibodies that target surface antigens on spermatozoa, impairing sperm function through agglutination, immobilization, opsonization, and the inhibition of sperm-oocyte interaction (Bohring & Krause, 2003; Liu *et al.,* 1987). These antibodies can be generated in both men and women, though the mechanisms differ. In men, ASAs are typically produced following the disruption of the blood-testis barrier due to trauma, infection, or surgery, particularly vasectomy (Shibahara *et al.,* 2020). In women, exposure to sperm antigens through sexual activity especially under conditions of genital tract inflammation or mucosal breach can lead to sensitization and subsequent ASA production (Bronson, 2001; Haas, 1986).

The immunoglobulin class of these antibodies plays a critical role in their functional implications. IgA antibodies, commonly found at mucosal sites, may interfere with sperm motility and prevent their progression through cervical mucus. IgG antibodies, the most abundant in systemic circulation, can fix complement and reduce sperm viability. IgM antibodies, although less frequently detected, are potent agglutinins and can mediate cytotoxic responses (Bronson, 2001; Kutteh, 1999). Differentiating among these immunoglobulin classes is essential to understanding the pathophysiology of immune infertility and tailoring appropriate therapeutic interventions.

Despite significant research into ASAs in Western populations, data from sub-Saharan Africa remain limited. This represents a critical knowledge gap, as regional factors such as genetic diversity, infectious disease burden, reproductive health practices, and healthcare accessibility may influence both the prevalence and immunological characteristics of ASAs (Okonofua, 2003). In Nigeria, where infertility carries profound sociocultural implications, immunological assessments are rarely included in standard infertility workups, leading to underdiagnosis and inappropriate management.

Moreover, while previous studies have established the presence of ASAs, few have examined their immunoglobulin class distribution, and even fewer have done so in relation to gender differences. Understanding whether men and women differ in the types of ASAs they produce—and the clinical relevance of those differences could enhance diagnostic precision and inform gender-specific treatment strategies (Francavilla *et al.,* 2007; Clarke *et al.,* 2006).

In this context, the present study investigates the prevalence and immunoglobulin class distribution (IgA, IgG, and IgM) of antisperm antibodies among infertile males and females in Edo State, Nigeria. By examining sex-specific differences in antibody class expression, this research aims to address the paucity of regional data and provide insights into the immunological underpinnings of infertility in this population. The findings are expected to contribute to improved clinical screening and the development of more targeted, evidence-based interventions for immune-mediated infertility.

**2.0 MATERIALS AND METHODS**

**2.1 Study Design and Location**

This study utilized a **descriptive cross-sectional design** to assess the distribution of antisperm antibody (ASA) immunoglobulin classes IgA, IgG, and IgM in infertile men and women in **Edo State, Nigeria**. The study was conducted across multiple fertility clinics and diagnostic centers within Benin City and its environs, chosen due to their high client turnout for infertility evaluations. The research period spanned **nine months (December 2024 to March 2025)** to allow adequate sample size accrual and laboratory processing.

**2.2 Study Population and Sampling Criteria**

The study included **96 consenting infertile individuals** (28 males and 68 females), aged **18 to 45 years**, attending infertility clinics. Infertility was defined following the WHO criteria as the **inability to conceive after 12 months of regular, unprotected sexual intercourse** (WHO, 2020). Participants were selected using **purposive sampling**, focusing on individuals who had tested positive for ASAs during preliminary screening.

Inclusion criteria:

1. Diagnosed with infertility
2. Positive result for ASA via slide latex agglutination test
3. Not currently pregnant or on immunosuppressive therapy
4. No history of autoimmune disorders

Exclusion criteria:

1. Acute systemic or genitourinary infections
2. Recent pelvic or scrotal surgery (within 6 months)
3. Prior vasectomy (in males)

Demographic information and relevant clinical history were collected through structured interviewer-administered questionnaires and clinical records.

**2.3 Blood Sample Collection and Handling**

Venous blood (5 mL) was collected aseptically from each participant using sterile plain vacutainer tubes. Samples were transported in cold-chain conditions (2–8°C) to the laboratory within 1–2 hours of collection. Blood was allowed to clot at room temperature for 30 minutes before being centrifuged at **3000 rpm for 10 minutes**. The **serum was separated and stored at –20°C** in aliquots to prevent repeated freeze-thaw cycles, which could degrade immunoglobulin proteins (Burtis *et al.,* 2020).

**2.4 Detection of Antisperm Antibodies**

Preliminary ASA detection was performed using the **Slide Latex Agglutination Test (SLAT)**, a qualitative screening method that detects sperm-bound or circulating antibodies via visible agglutination. This test involves mixing patient serum with latex particles coated with sperm antigens. Agglutination indicates the presence of ASAs. The SLAT method was chosen for its **cost-effectiveness, simplicity, and rapid results** in clinical settings (Friberg & Sjöblom, 1982).

Only participants who tested positive by SLAT were further analyzed for immunoglobulin class differentiation.

**2.5 Determination of Immunoglobulin Class of ASAs**

To identify specific antibody classes, the study employed the **Antispermatozoa Antibody ELISA Ig-Classifying Test Kit** (Demeditec Diagnostics, Germany), which quantitatively detects IgA, IgG, and IgM classes of ASAs in serum. This test follows the principles of **indirect ELISA**, where sperm antigens coated on microtiter plates bind to class-specific antibodies in the patient’s serum.

The ELISA procedure involved the following steps:

1. **Incubation** of serum samples in antigen-coated wells for 30 minutes at 37°C.
2. **Washing** to remove unbound components.
3. **Addition of enzyme-labeled anti-human IgA, IgG, or IgM conjugates**.
4. **Second incubation** followed by washing.
5. **Substrate addition** (TMB chromogen) and incubation to allow color development.
6. **Stopping the reaction** and reading absorbance at **450 nm** using a microplate spectrophotometer.

Optical density (OD) values were interpreted based on cut-off thresholds provided by the manufacturer. Samples with OD values above the reference threshold were considered positive for the corresponding immunoglobulin class. The manufacturer’s cut-off values were as follows: for IgA > 10 U/mL, IgG > 10 U/mL, and IgM > 10 U/mL. Samples exceeding these thresholds were interpreted as positive. Borderline values (8–10 U/mL) were re-tested to confirm results, and only consistent positives were included in the final analysis to reduce the risk of false positives. The manufacturer’s cut-off values were as follows: for IgA > 10 U/mL, IgG > 10 U/mL, and IgM > 10 U/mL. Samples exceeding these thresholds were interpreted as positive. Borderline values (8–10 U/mL) were re-tested to confirm results, and only consistent positives were included in the final analysis to reduce the risk of false positives.

**2.6 Quality Control and Validation**

All assays were performed in duplicate, and positive and negative controls provided by the manufacturer were included in every run to ensure analytical validity. Internal laboratory quality control procedures followed **CLSI (Clinical and Laboratory Standards Institute)** guidelines (CLSI, 2014). ELISA kits were stored at 2–8°C and used before their expiration date.

**2.7 Statistical Analysis**

Data entry and analysis were conducted using **IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY)**. Descriptive statistics such as means, standard deviations, frequencies, and percentages were used to summarize demographic and laboratory variables. The **Chi-square test (χ²)** was used to determine associations between immunoglobulin class distribution and sex of participants. A p-value of **< .05 was considered statistically significant**. Results were presented using tables and bar charts for visual clarity and this method has be used by Atemoagbo *et al* (2024). To further explore associations, Cramér’s V was calculated to estimate effect sizes for significant Chi-square results, with values interpreted as small (0.1), medium (0.3), or large (0.5) effects. Additionally, binary logistic regression was used to evaluate the predictive power of sex on the presence of each immunoglobulin class while controlling for age and duration of infertility

**3.0 RESULT AND DISCUSSION**

**3.1 Results**

A total of 96 participants (28 males and 68 females) who tested positive for antisperm antibodies (ASAs) were further assessed for immunoglobulin class distribution. The ELISA-based immunoglobulin classification revealed significant variation in the distribution of IgA, IgG, and IgM classes across genders.

**3.1.1 Immunoglobulin Class Distribution by Gender**

As shown in Figure 1, females exhibited a statistically significant increase in the prevalence of IgA (72.2% vs. 27.8%, p < .01) and IgM (73.9% vs. 26.1%, p < .05) compared to males. In contrast, IgG prevalence did not differ significantly between females (53.6%) and males (46.4%, p = .251).

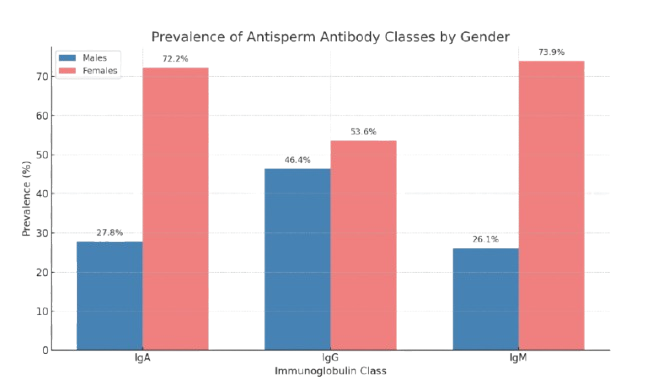


Fig 1- **Prevalence of Antisperm Antibody Classes based on Gender**

**3.1.2 Statistical Analysis**

Chi-square tests of independence were used to assess the significance of sex-based variation in immunoglobulin class prevalence. Results are summarized as follows:

1. **IgA**: χ² = 15.47, **p < .01** — significant difference.
2. **IgM**: χ² = 8.67, **p < .05** — significant difference.
3. **IgG**: χ² = 1.32, **p = .251** — not significant.

These results demonstrate that IgA and IgM classes are significantly more common among females, while IgG exhibits no sex bias.

**3.2 Discussion**

The findings from this study illuminate significant sex-based variations in the distribution of antisperm antibodies (ASAs), particularly with respect to IgA and IgM classes, among infertile individuals in Edo State, Nigeria. These findings not only align with global literature but also fill a critical gap in African-based immunological infertility studies.

**3.2.1 Biological and Immunological Interpretation**

The predominance of **IgA ASAs in females (72.2%)** may reflect the mucosal immune activity of the **female reproductive tract**, where secretory IgA plays a critical role in local immune defense (Mestecky *et al.,* 2005). Since sperm antigens are introduced repeatedly through coitus, **female mucosal surfaces are continuously exposed**, promoting an IgA-mediated response (Groot *et al.,* 2016). This localized immunity may escalate into systemic circulation, explaining elevated serum IgA levels.

**IgM**, typically indicative of an early immune response, was also significantly higher in females. This could suggest recurrent or recent **reproductive tract infections** that contribute to antigen exposure and immune activation. Unlike IgA and IgG, IgM is pentameric and generally does not penetrate tissues efficiently; however, its detection in serum still signifies immunogenic activity against spermatozoa (Shibahara *et al.,* 2020).

In contrast, **IgG levels were similar in both sexes**, a pattern consistent with systemic immune responses often resulting from **chronic exposure or memory B-cell activation** (Bronson & Fusi, 1997). IgG antibodies are implicated in impairing sperm motility and interaction with cervical mucus, regardless of gender (Atemoagbo, 2024).

**3.2.2 Comparison with Previous Literature**

Our findings are consistent with prior studies from Europe and Asia. Bohring and Krause (2003) reported that IgA and IgG ASAs were predominant in female infertility, often linked to failure in sperm penetration and fertilization. Talwar *et al.* (2007) also reported higher titers of IgA among infertile Indian women. However, data from African populations remain scarce, making this study a unique contribution.

The similarity in IgG prevalence across sexes also reflects earlier observations by Naz (2014), who noted that **IgG ASAs have comparable fertility-damaging potential** in both males and females due to their ability to traverse tissue barriers and interfere with fertilization mechanisms.

**3.2.3 Sex-Based Immunological Differences**

The **higher immune reactivity in females** is supported by broader immunological research indicating that women have stronger **humoral immune responses**, likely due to genetic and hormonal differences (Klein & Flanagan, 2016). Estrogen enhances B-cell activation and antibody production, while testosterone tends to be immunosuppressive. This immunological dimorphism may partly explain the higher detection of IgA and IgM ASAs in female participants.

**3.2.4 Clinical Implications**

These findings have immediate relevance for clinical fertility management:

* **IgA and IgG ASAs** have been linked to impaired sperm motility, agglutination, and cervical mucus penetration—critical in both natural conception and assisted reproduction (Bronson & Fusi, 1997; Naz, 2014).
* **Presence of IgM**, while less likely to cause direct sperm dysfunction, may signal ongoing antigen exposure and inflammation, warranting further investigation for underlying infections.

In clinical settings, **screening for ASA subclasses using ELISA** can aid in determining treatment plans. For instance, patients with high IgA or IgG titers may benefit more from **assisted reproductive techniques** such as intrauterine insemination (IUI) or in vitro fertilization (IVF), possibly combined with **immunosuppressive therapies** (Naz, 2014).

**3.2.5 Study Limitations**

Despite its contributions, this study has some limitations. First, the cross-sectional design limits causal inference; longitudinal studies would better assess temporal changes in ASA profiles. Second, the sample size, though adequate for exploratory analysis, may limit generalizability, particularly for the male subgroup. Third, the reliance on purposive sampling from fertility clinics may introduce selection bias, as participants likely represent a population already seeking care. Additionally, the study did not assess potential confounding factors such as hormonal status, infection history, or exposure to environmental toxins, which could influence antibody production. Finally, while the ELISA test used was validated, the absence of functional assays (e.g., sperm-penetration tests) limits insight into the actual impact of ASAs on fertility outcomes. Future studies incorporating broader immunological markers and larger, multi-center cohorts are recommended to strengthen the clinical applicability of these findings.

**4.0 Conclusion and Recommendations**

**4.1 Conclusion**

This study provides novel insights into the immunoglobulin class distribution of antisperm antibodies (ASAs) among infertile males and females in Edo State, Nigeria, highlighting significant sex-based differences in immune-mediated infertility. The results demonstrate that IgA is the most prevalent ASA class overall, with a significantly higher incidence in females (72.2%) compared to males (27.8%, p < .01). Similarly, IgM antibodies were more frequent in females (73.9%) than males (26.1%, p < .05), suggesting a pronounced mucosal and early immune response in women. In contrast, IgG prevalence showed no significant sex difference (p = .251), indicating its role as a systemic antibody with comparable impact across genders. These findings underscore the critical influence of gender-specific immune responses in the pathophysiology of infertility, likely driven by differences in reproductive tract anatomy, hormonal regulation, and exposure to sperm antigens.

The higher prevalence of IgA and IgM in females aligns with their mucosal immunity and potential for recurrent antigenic stimulation, possibly exacerbated by local infections or inflammation. The lack of sex disparity in IgG prevalence reflects its systemic distribution and chronic immune activation, consistent with its established role in impairing sperm function across both sexes. These patterns not only corroborate global trends but also address a significant research gap in sub-Saharan Africa, where immune infertility remains understudied despite its sociocultural and clinical relevance.

From a clinical perspective, the predominance of IgA in females suggests that mucosal immune responses may disproportionately contribute to female infertility in this population, potentially through mechanisms such as sperm immobilization or cervical mucus penetration failure. The elevated IgM in females further hints at ongoing or recent immunological triggers, warranting investigation into underlying inflammatory or infectious etiologies. Meanwhile, the consistent IgG presence across genders emphasizes its universal relevance in immune infertility, likely affecting sperm viability and fertilization potential.

This study’s findings have broader implications for understanding immune infertility in diverse populations. By delineating immunoglobulin class profiles, it lays the groundwork for refining diagnostic approaches and therapeutic strategies tailored to the immunological characteristics of affected individuals. Moreover, it highlights the need for region-specific research to account for genetic, environmental, and healthcare-related factors that may modulate ASA prevalence and impact.

**4.2 Recommendations**

Based on the results of this study, the following recommendations are proposed for clinical practice, research, and policy, suitable for consideration in high-impact journals:

* 1. Routine Immunological Screening in Infertility Workups: Fertility clinics in Nigeria and similar settings should incorporate ASA screening, particularly IgA and IgG subclass identification via ELISA, into standard diagnostic protocols for unexplained infertility. This would enhance the detection of immune-mediated causes, especially in females where IgA predominates, and guide more precise interventions.
  2. Gender-Specific Treatment Approaches: Given the higher prevalence of IgA and IgM in females, treatment strategies for women with immune infertility should prioritize addressing mucosal immunity and potential inflammatory triggers. In contrast, the consistent IgG presence across sexes supports the use of assisted reproductive technologies (ART) such as intrauterine insemination (IUI) or in vitro fertilization (IVF) for both men and women with elevated ASA titers. Adjunctive immunosuppressive therapies, such as corticosteroids, could be explored in cases with high IgA or IgG levels, pending further clinical trials.
  3. Investigation of Underlying Triggers: The significant IgM prevalence in females suggests possible recent or ongoing antigenic stimulation, potentially linked to reproductive tract infections. Clinicians should investigate and manage concurrent genital infections (e.g., bacterial vaginosis, sexually transmitted infections) as part of infertility treatment plans to reduce ASA production and improve outcomes.
  4. Further Research in African Populations: This study underscores the scarcity of data on immune infertility in sub-Saharan Africa. Future research should expand to other regions, employing larger sample sizes and longitudinal designs to validate these findings and explore additional factors (e.g., genetic polymorphisms, environmental exposures) influencing ASA distribution. Comparative studies across African and non-African cohorts could further elucidate population-specific immunological profiles.
  5. Development of Targeted Immunotherapies: The distinct immunoglobulin profiles identified here suggest potential for developing class-specific interventions, such as IgA-neutralizing agents or mucosal immunomodulators, to mitigate ASA effects in infertile patients. Collaborative efforts between immunologists and reproductive specialists are needed to translate these findings into viable therapeutic options.
  6. Public Health and Awareness Initiatives: In Nigeria, where infertility carries significant social stigma, health authorities should promote awareness of immune infertility as a treatable condition. Integrating ASA testing into public health frameworks could reduce misdiagnosis and improve access to specialized care, particularly in resource-limited settings like Edo State.

In conclusion, this study advances the understanding of immune infertility by delineating the immunoglobulin class distribution of ASAs in a Nigerian cohort, with clear implications for personalized medicine and public health. By addressing these recommendations, stakeholders can enhance diagnostic accuracy, optimize treatment efficacy, and ultimately alleviate the burden of infertility in affected populations. These insights merit further exploration and validation to inform global reproductive health strategies.

**Ethical Approval and consent**

Approval for the study protocol was obtained from the **Edo State Ministry of Health Research Ethics Committee**, in accordance with the **Declaration of Helsinki on ethical principles for medical research involving human subjects** (World Medical Association, 2013). All participants received comprehensive information about the study objectives, procedures, potential risks, and benefits, and provided **written informed consent** before sample collection.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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