

SPERMATOZOAL, SEMINAL PLASMA AND BLOOD SPERM ANTIBODIES IN NIGERIAN MALES

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SUMMARY

Infertility is common in Nigeria and the male role is now well recognized. Sperm antibodies (SA) have been observed as a cause of infertility and their production have in some circumstances been attributed to chronic infection of the genital tract. Sexually transmitted diseases (STDs) and infection-related infertility are reportedly highly prevalent in Nigeria. This study was therefore designed to evaluate the role of sperm antibodies and determine the involvement of STDs in the production of such SA among Nigerians.

122 adult males aged 18-56 years were investigated. 25 were normospermic, fertile males with no evidence of STDs and served as controls. 50 were infertile without STDs while 47 had proven STDs. Immunobead binding technique was used for the detection of SA directly on the sperm cell, in seminal and blood plasma. Student's *t*-test and anova (one-way) were used for statistical analysis of data obtained.

Results showed that SA- IgG, IgA and IgM were present in blood and semen. However, mean percentage binding of these SA on motile sperm was low (<4%). Comparisons of SA in blood and semen between infertile/STDs groups and fertile controls were not significantly different ($p>0.05$)

The findings suggest that sperm antibodies are present but may not be associated with STDs or infertility in Nigerian males.

Keywords: Sperm antibodies, male infertility, sexually transmitted diseases, blood, seminal plasma, spermatozoa.

INTRODUCTION

Infertility is a medico-social problem in the world over including sub-Saharan Africa (1,2). The currently reported high incidence of male infertility mandates the evaluation of male partners of couples consulting for infertility (3). Knowledge of male infertility is limited (4) and proof of the ability of normospermic men to fertilise remains indirect (5).

Sperm antibodies (SA) have been shown to have adverse effect on fertility. Studies suggest that male autoimmunity is more prevalent than female isoimmunity and a woman's isoimmunity is often associated with her husband's autoimmunity. (6,7) However, the role of SA in male infertility remains controversial (8) and is a subject of interest worldwide. (7)

Several risk factors including genital tract infections have been defined for the development of SA. (9) Genital inflammation facilitates the formation of SA. (10). Past or chronic infection of the genital tract especially 'silent' infection of *Chlamydia trachomatis* (*C. trachomatis*) have been implicated (11). In sub-Saharan Africa, sexually transmitted diseases (STDs) especially *Neisseria gonorrhoea* (*N.gonorrhoea*) and *C.trachomatis* are common place. (12)

Infection related infertility is alarmingly high

in Africa (13,14). Many infertile men have STDs especially gonorrhoea and non-specific urethritis (NSU) (15).

Several investigators observed the incidence of SA in association with STDs and reduced fertility in men (16,17,18). Ekwere in his study in sub-Saharan Africa, reported a high incidence (44%) of sperm antibodies in association with STDs among infertile men (19).

Detection of immunoglobulin class and localization of antibodies on the spermatozoa can be of clinical, diagnostic and prognostic value (20,21). IgG, IgA and IgM antibody isotypes have been observed as the most common SA in serum, seminal plasma and bound to sperm (22,23,24).

Traditionally, the identification of patients with SA has depended on tests in blood serum. This has been criticized and there is growing emphasis on the investigation of local rather than systemic immunity to spermatozoa, since serum antibodies are not necessarily an indication of the antibody activity in seminal plasma and on the sperm membrane. (25) SA identified directly on sperm surface reportedly correlate best with the presence of antibody-mediated infertility (9).

This study therefore was designed to comprehensively investigate and explore the probable role of SA as well as determine the involvement of STDs in the production of such antibodies in Nigerian males.

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MATERIALS AND METHODS

Subjects

A total of 122 Nigerian males aged 18-56 were recruited under three groups-fertile subjects (control), infertile subjects and subjects with STDs. All subjects gave informed consent. Fertility was based on a satisfactory semen profile and achievement of at least one pregnancy. Both fertile and infertile had no STDs while the STDs group was investigated before treatment.

Sample Collection

Blood, semen, and urethral swab were obtained from subjects. Venous blood was collected in lithium heparin tubes, centrifuged for 5 minutes at 3000rpm. Plasma was collected and stored at -20°C for analysis. Semen was obtained from the subjects by masturbation after abstinence from sexual activity for 3 days and analysis performed biophysically according to World Health Organisation guidelines (26). Seminal plasma was obtained by centrifuge at 3000rpm for 30 mins and seminal plasma obtained was stored at -20°C for detection of SA. Urethral swab for microbiological analysis was collected from urethra of each male subjects by rotating swab approximately for 5 seconds after inserting 2 to 4 cm into the urethra.

Immunological Analysis

IgG, IgA and IgM SA were detected on sperm cell using direct immunobead binding technique (IBT) while indirect IBT was used to detect IgG, IgA, and IgM SA in blood plasma and seminal plasma (27). Immunobeads were obtained from Biorad, Richmond C.A.

Diagnosis of STDs

All STDs diagnosis were made first on clinical grounds by a consultant microbiologist at the STDs clinic at the UCH and confirmed by laboratory tests except for lymphogranuloma venereum, genital wart, herpes, genital ulcers and tinea cruris which were diagnosed on clinical grounds only (28,29).

Statistical Analysis

Statistical analysis were carried out by means of computer statistical soft ware-Epi-info 6.02. Student's t-test (paired and unpaired) and analysis of variance (anova)-one way were used for comparison of means.

RESULT

Sperm antibodies

Spermatozoal SA (X)

Mean percentage binding of X-IgG, X-IgA and X-IgM were low ranging from 1.2% -4.1% in all groups tested (Table 1). No significant difference

Table 1: Mean(\pm s.e) percentage binding of sperm antibodies – IgG, IgA, IgM to Spermatozoa , Seminal plasma, Blood plasma and incidence of men with these sperm antibodies in fertile, infertile and STDs groups.

Sperm antibody	Fertile			Infertile			STDs			p
	Spermatozoal	Seminal plasma	Blood plasma	Spermatozoal	Seminal plasma	Blood plasma	Spermatozoal	Seminal plasma	Blood plasma	
IgG	n=21 3.3 \pm 1.2 1(4.7)*	n=24 1.2 \pm 0.4 0(0)*	n=23 0.7 \pm 0.2 0(0)*	n=18 2.8 \pm 0.7 0(0)*	n=39 1.4 \pm 0.8 1(2.6)*	n=45 2.0 \pm 0.4 0(0)*	n=7 4.1 \pm 3.5 1(14.3)*	n=14 1.1 \pm 0.4 0(0)*	n=43 1.3 \pm 0.3 0(0)*	>0.05
IgA	n=21 3.8 \pm 0.8 0(0)*	n=24 2.2 \pm 1.2 1(4.2)*	n=23 0.7 \pm 0.3 0(0)*	n=17 1.2 \pm 0.7 0(0)*	n=39 1.6 \pm 0.3 0(0)*	n=45 1.2 \pm 0.2 0(0)*	n=5 2.4 \pm 1.7 0(0)*	n=14 1.0 \pm 0.3 0(0)*	n=43 1.7 \pm 0.4 0(0)*	>0.05
IgM	n=21 2.3 \pm 2.8 0(0)*	n=21 1.3 \pm 0.2 0(0)*	n=23 0.5 \pm 0.2 0(0)*	n=16 2.7 \pm 0.6 0(0)*	n=39 1.5 \pm 0.5 1(2.6)*	n=45 1.6 \pm 0.4 0(0)*	n=5 1.2 \pm 0.7 0(0)*	n=14 1.1 \pm 0.5 0(0)*	n=14 1.3 \pm 0.3 0(0)*	>0.05

*=Incidence-values are in proportions with percentages in parentheses; STDs = sexually transmitted diseases; s.e = standard error; p =probability; n= number of subjects.

was observed in the comparison of mean percentage binding of X-IgG between fertile, infertile and STDs groups ($p>0.05$) Similar observations were made in X-IgA and X-IgM in comparisons between fertile, infertile and STDs subjects. Comparisons between normospermic (BN) and dyspermic (BA) infertile males did not

reveal significant differences in X-IgG, X-IgA and X-IgM ($p>0.05$)(Table2).

The incidences of men with X-IgG, X-IgA and X-IgM varied between the different groups studied ranging from 0% - 14.3% (Table 1).

Tail binding was observed in all isotypes of X in all groups tested.

Table 2: Mean(\pm s.e) percentage binding of sperm antibodies – IgG, IgA, IgM to spermatozoal, seminal plasma and blood plasma, and incidence of men with these sperm antibodies in normospermic and dyspermic infertile men .

Sperm antibody	Normospermic Infertile men			Dyspermic Infertile men			p
	Sperma-tozoal	Seminal plasma	Blood plasma	Sperma-tozoal	Seminal plasma	Blood plasma	
IgG	n=8 3.0 \pm 1.1 0(0)*	n=10 1.4 \pm 1.2 0(0)*	n=13 1.9 \pm 1.0 0(0)*	n=10 2.6 \pm 1.0 0(0)*	n=29 1.4 \pm 1.0 1(3.4)*	n=32 2.1 \pm 0.5 1(3.4)*	>0.05
IgA	n=8 1.1 \pm 0.6 0(0)*	n=10 1.0 \pm 0.6 0(0)*	n=13 1.2 \pm 0.3 0(0)*	n=9 1.6 \pm 1.3 0(0)*	n=29 1.8 \pm 0.4 0(0)*	n=32 1.2 \pm 0.3 1(3.4)*	>0.05
IgM	n=8 2.4 \pm 0.7 0(0)*	n=10 0.8 \pm 0.3 0(0)*	n=13 1.5 \pm 0.4 0(0)*	n=8 3.0 \pm 1.0 0(0)*	n=29 1.8 \pm 0.7 1(3.4)*	n=32 1.7 \pm 0.5 0(0)*	>0.05

*=Incidence-values are in proportions with percentages in parentheses; STDs = sexually transmitted diseases; s.e = standard error; p =probability; n= number of subjects.

Seminal Plasma SA (Y)

The mean percentage binding of Y-IgG, Y-IgA and Y-IgM were also low ranging from 1.0%-2.2% in all groups tested (Table 1). No significant difference was observed in comparison of mean percentage binding of Y-IgG between fertile, infertile and STDs groups ($p>0.05$). Comparisons between normospermic (BN) and dyspermic infertile males (BA) did not reveal significant differences in Y-IgG, Y-IgA and Y-IgM ($p>0.05$)(Table2).

The incidences of men varied between the different groups studied ranging from 0%-4.2% in Y-IgG, Y-IgA and Y-IgM (Table 1).

Unlike X,Y bound to different regions of sperm cell. However, tail and head binding were more commonly observed in all groups.

Blood plasma SA(Z)

Similar to X and Y, the mean percentage binding of Z-IgG, Z-IgA and Z-IgM were low ranging from 0.5%-2.0% in all groups tested (Table 1). Comparison of mean percentage binding of Z-IgG showed no significant difference between fertile, infertile and STDs groups ($p>0.05$). Similar observations were made in comparisons between fertile, infertile and STDs subjects in Z-IgA and Z-IgM. Comparisons between normospermic and dyspermic infertile males did not reveal significant differences in Z-IgA and Z-IgG, Z-IgM ($p>0.05$)(Table 2).

The incidence of men with Z-IgG, Z-IgA, Z-IgM was zero in the different groups studied (Table1).

Binding of Z were observed on the different regions of the sperm cell – head, tail and tail tip. These antibodies bound to tail and tail tip more

commonly in all groups tested-fertile, infertile and STDs groups.

SA isotypes

Comparison of binding percentage of SA between the different classes of X-IgG, IgA and IgM in fertile group did not reveal any difference that was significant ($p>0.05$). Similar observations as made in infertile and STDs groups.

Similarly comparisons of binding percentage of SA between classes-IgG, IgA and IgM of Y and Z in fertile, infertile and STDs groups did not show any significant differences ($p>0.05$).

SA location

IgG SA were not significantly different between the three body locations-X,Y and Z ($p>0.05$). Neither were there significant differences in IgA and IgM SA ($p>0.05$).

Discussion

Similar to findings of other investigators (22, 23, 30), IgG, IgA and IgM SA were demonstrated in blood plasma, seminal plasma and on spermatozoa.

Studies on immunoglobulin class of SA have not been consistent in findings. IgG was observed as the most predominant immunoglobulin in sera and semen (31,32). Combaire observed that secretory IgA does occur in semen and not serum (33). Systemic inoculation of sperm antigens is thought to stimulate IgG production whereas local reproductive tract antigens are stimuli for IgA production; thus suggesting a primary, locally induced antibody of IgA type which may be harmful than a secondary, systemically produced IgG antibody. Other studies (22, 34,35) have observed IgA more commonly than IgM. IgM are large molecules and are confined to serum and only rarely found in organs and

secretions of male genital tract. (9). Hence IgM SA are not routinely measured in detection systems because their role in antibody mediated infertility is presumably limited (30).

In this study, binding proportions of IgG SA were similar to IgA and IgM SA in all body locations and groups tested ($p>0.05$). These findings do not show a significantly higher level of binding of one antibody class over the other classes in all the body locations-spermatozoa, seminal plasma and blood plasma. This indicates the possibility of both systemic and local production of SA-IgG, IgA and IgM. IgM may like IgG and IgA have a role in responding to sperm-antigenic challenge. A new approach in including the measurement of IgM to IgG and IgA in semen and serum may be worthwhile in resolving the existing conflicts of their role in infertility. Moreover either of the immunoglobulin classes can be measured in detection systems thus minimising cost of antibody testing.

Sperm-bound antibodies are said to be the appropriate to measure for sperm functional capacity because sperm alone reach the female reproductive tract and the sperm plasma membrane remains intact in sperm participating in in-vivo fertilization until after the acrosome reaction (9, 30,36). It was argued that serum SA cannot logistically bind to sperm unless they transudate into semen, hence they are considered clinically less important than sperm-bound antibodies.

Comparisons of IgG SA between different locations – spermatozoa, seminal plasma and blood plasma did not show any significant differences in all groups ($p>0.05$). Similarly, comparisons between X-IgA, Y-IgA and Z-IgA did not reveal any significant differences in all groups ($p>0.05$). Neither were there significant differences in the comparisons between X-IgM, Y-IgM and Z-IgM in all groups tested ($p>0.05$). These observations further confirms the local and systemic production of SA but contrasts the more clinical relevance of spermatozoal SA over serum SA. A positive correlation between SA in blood plasma and seminal plasma has been observed (37). Moreover, 50% - 80% of vasectomized males (the most thoroughly studied association with SA) have measurable levels of SA in serum and rarely in seminal plasma (30,32).

Clinically, antibodies to sperm are found in 3% to 12% of infertile men (30,38,39). Similar variations were observed in this study. The incidences of men with X,Y and Z varied between groups and classes ranging from 0%-14.3%(Table1). Similar discrepancies have been observed in other studies (23,31,37,40,41).

The presence of immunoglobulin isotypes – IgG, IgA and IgM SA in blood and semen and the

similarity of incidences of men with these antibodies between this study and others (30) is suggestive of their possible role in male infertility. However, such a role is less obvious due to the finding of low (4.1%) percentage binding in SA in all antibody isotypes, in all body locations and in all groups (Table1) in this present study. SA percentage binding of less than 60% for IgG and less than 40% for IgA were observed in all cases with proven fertility (35). It was reported that sperm bound antibody of less than 50% need not be treated since post-coital tests approximate fertile controls (30). It was also suggested that the failure of intrauterine insemination in the treatment of male immunological infertility is imputable to SA when they involve all spermatozoa regardless of sperm quality (42).

Differences exist in the region of binding of SA. Head, tail and tail tip binding of SA have been observed (30,27,43,44). Similar observations were made in this present study. Head, tail and tail tip binding of X, Y and Z were observed in all groups – fertile, infertile and STDs groups. However X preferentially bound to tail, Y to head and tail while Z preferentially bound to tail and tail tip regions irrespective of immunoglobulin class and group. These observations were contrary to findings in other studies where specific immunoglobulin classes have been associated with specific regions of binding (27,43,45). The effect of sperm antibodies on sperm function depends on the area to which the antibodies are directed (46). Tail directed antibodies only weakly affect cervical mucus interactions. It was therefore suggested that tail directed SA need not be treated since it is thought that only head-directed or midpiece directed SA are clinically relevant in immobilisation or penetration assays³⁰. It was also observed that antibodies that are attached to sperm head with binding rate of less than 40% were not considered to be causative factor of male infertility (35). Head directed sperm antibodies inversely correlated with zona adherence, are said to be more potent blocks of normal gamete interactions than are tail directed antibodies. Sperm-zona binding via specific receptors could be blocked by steric interference from attached head-directed antibodies thus affecting fertility at the level of gamete interaction (40). Antibodies to tail tip rarely have any effect on sperm function (41,27). The finding of head-directed SA in seminal plasma in this present study may be important but antibody binding rates are far too low to affect fertility. Moreover, this observation is similar in all groups-fertile, infertile and STDs groups. These findings in addition to the observation of tail binding as the most prominent region of binding of sperm antibodies further support the fact that sperm antibodies may not affect fertility in males.

In order to further elucidate this conflict, comparisons of SA between test groups and controls

were carried out in this present study. Comparisons of X-IgG between infertile group and fertile controls did not show any significant difference ($p>0.05$). Neither was the difference between STDs and fertile controls significant ($p>0.05$). Further comparisons within the infertile group – between normospermic infertile males and dyspermic infertile males also showed no significant differences in X-IgG, X-IgA and X-IgM ($p>0.05$). Similar comparisons as X above in Y-IgG, Y-IgA and Y-IgM and Z-IgG, Z-IgA and Z-IgM did not show differences that were significant ($p>0.05$). These observations were also reported by others (48,49). Comparisons of seminal plasma SA showed no significant difference between infertile and fertile men as well as between genitourinary tract infection and the formation of seminal SA (48). Neither was there significant enhancement in serum levels of antibodies in a similar study (49).

It is probable that SA though present do not have a role in male infertility judging from the observations made in this study. Similarly, the production of SA may not be influenced by STDs. Thus, the diagnosis of immunological infertility still remains one of exclusion (30). A study carried out in Benin-city, Nigeria, a similar geographical sub-region as this present study amongst infertile women did not relate infertility to the presence of sperm antibodies (50).

CONCLUSION

Sperm antibodies are present but may not be a factor of infertility in Nigerian males. STDs may have no role in the production of these SA.

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