East African Medical Journal Vol. 94 No. 2 February 2017
SERO-PREVALENCE OF ANTI-SPERM ANTI-BODIES IN INFERTILE MALES IN PORT HARCOURT, NIGERIA
I.N. Emeghe, DVM, Department of Haematology, Blood Transfusion and Immunology and O. N. Ekeke, MB.BS, FWACS, FICS, Consultant Urologist, Department of Surgery, University of Port Harcourt, Nigeria.

Request for reprints to: Dr. O.N. Ekeke, Department of Surgery, University of Port Harcourt, P. O. Box. 10697, Port Harcourt, Nigeria.

SERO-PREVALENCE OF ANTI-SPERM ANTI-BODIES IN INFERTILE MALES IN PORT HARCOURT, NIGERIA

I.N. EMEGHE and O. N. EKEKE,

ABSTRACT

Background: **Infertility is a serious health challenge which causes distress to the couples especially; in Africa. The cause of infertility is multifactorial. Immunological infertility is said to be one of the major causes of unexplained infertility in men. Anti-sperm anti-bodies can be used as an immunological marker of infertility.**
Objective: **To determine the sero-prevalence of anti-sperm anti-bodies in Port Harcourt.**
Design: **A cross sectional study.**
Setting: **Three hospitals in Port Harcourt, Nigeria.**
Subjects: **Ninety men who presented with infertility.**
Outcome: **Presence of anti-sperm anti-bodies.**
Results: **The results obtained showed that the modal age group for infertile males was 40-49 years. Mean duration of infertility was 5.54±2.34 years. Sixty nine patients (76.67%) had primary infertility, 60% had previous sexually transmitted infections, 65.23% had low sperm counts, 88.46% had abnormal sperm motility and anti-sperm anti-bodies were detected in 26.67%. Presence of anti-sperm anti-bodies were associated with age ≥ 40 years, previous history of sexually transmitted infections, duration of infertility, and abnormal sperm motility. There was no association between hormone profile and presence of anti-sperm anti-bodies.**
Conclusion: **Anti-sperm anti-bodies are fairly common in Port Harcourt; and immunological factors may contribute to infertility in male patients of the sub-region.**

INTRODUCTION

Infertility is defined as the inability of opposite sex couples to achieve pregnancy following one year of regular, unprotected sexual intercourse (1). It could be seen as a public health problem which has not only physical, but also social and psychological implications. The burden of infertility is gradually increasing and it accounts for over 50% of gynaecological consultations in a Nigerian clinic (2). Abarikwu reported a prevalence of 30% infertility among couples in Nigeria (3). There are few studies on unexplained infertility. Immunological infertility, due to the presence of anti-sperm anti-bodies, has been suggested to be the primary cause of unexplained infertility (4).

Immunologic infertility may be defined as an adverse immune response against sperm antigens that could cause sperm dysfunction. This immune response could be humoral or cellular (5).The formation of anti-sperm anti-bodies (ASAs) is a major feature in immunological infertility (4). The testis is an immune-privileged site and the sperm are protected from adverse immune reactions if they come in contact with the circulation by the blood-testis barrier. The release of sequestered sperm antigens on germ cells following a disruption of the blood-testis barrier (BTB) could lead to the development of ASAs (6). This breach in the BTB could occur during an operation such as a vasectomy, testicular torsion or an injury to the testis (7).

The prevalence of ASAs in infertile men varies across the globe (8) and it ranges from 5-44% depending on the test method used (9-16).

Although ASAs have been linked to unexplained infertility, tests for these anti-bodies are not yet available in our region; the literature on the subject is scarce in Nigeria. We therefore decided to undertake this study to evaluate the possible presence of ASAs in our infertile males as well as the factors associated with their presence.

MATERIALS AND METHODS.

The study was carried out in three centres in Port Harcourt: University of Port Harcourt Teaching Hospital (UPTH), Potter's Touch Medical Consultants (PTMC) and Paragon Clinics. It was a cross-sectional study. Serum specimens were obtained from all the male patients with complaints of infertility at the aforementioned hospitals within the period of study. Included were men married to the opposite gender that have been unable to impregnate their wives after a minimum of one year of unprotected regular coitus; excluded were other urology patients who did not complain of infertility. Participants were also interviewed with the aid of a pro forma. Information obtained included: age, type of infertility, duration of infertility, medical conditions that may affect fertility such as varicocoeles and mumps, sexually transmitted infections (STIs) and others.

Serum was extracted from clotted blood after centrifugation at 1,000 gyri and stored frozen at –200 C. Donor semen was collected by masturbation. Analysis of serum-anti-sperm anti-bodies were done by the Indirect SpermMar IgG Test (Fertipro N.V-Industrie park Noord 32-8730, Beernem, Belgium) using a commercial test kit according to manufacturer's instructions. The occurrence of 40% or more reaction between the coated latex particles and the motile spermatozoa is generally accepted as the lower limit of significant activity. Data were analysed using statistical package for social sciences (SPSS) ; (IBM SPSS Inc, Chicago, IL, USA, 2007, version 20.0). Chi-squared test of significance was used to compare proportions to test for association and p-value of <0.05 was considered statistically significant.

RESULTS

Out of the 90 infertile patients, the modal age group was 40-49 years. Sixty-nine patients (76.67%) had primary infertility while the mean duration of infertility was 5.54 + 2.34 years. Fifty-four patients (60%) had a history of previous infection with sexually transmitted infections (STIs). The socio-demographic characteristics are shown in table 1.

Table 2 displays the hormone profile of 80 of the participants. Most of the patients expressed normal hormone levels in their serum (83.75%) had normal FSH levels, 68.75% patients had normal levels of LH, 76.25% had normal level ranges of prolactin and 67.50% had normal ranges of testosterone

TABLE 1
Socio-demographic characteristics and history of infertile males

Characteristic	Frequency (n=90)	Percentage (%)
Age group (years)		
20-29	2	2.22
30-39	24	26.67
40-49	57	63.33
≥50	7	7.78
Mean Age	41.63 ± 6.64	
Type of Infertility		
Primary	69	76.67
Secondary	21	23.33
Duration of Infertility (years)		
1-2	14	15.56
3-4	39	43.33
5-6	23	25.56
≥7	14	15.56
Mean duration	5.54 ± 2.34	
History of previous STD		
Yes	54	60.00
No	36	40.00

TABLE 2
Hormone Profile of the infertile males

Characteristic	Frequency (n=80)	Percentage (%)
Follicle Stimulating Hormone (FSH) mIU/ml		
Upper Range (>11.5)	12	15.00
Normal Range (1.1-11.5)	67	83.75
Lower Range (<1.1)	1	1.25
Mean	8.31 ± 12.84	
Luteinising Hormone (LH) mIU/ml		
Upper Range (>7.8)	25	31.25
Normal Range (1.2-7.8)	55	68.75
Lower Range (<1.2)	0	0.0
Mean	8.67 ± 11.26	
Prolactin ng/ml		
Upper Range (>20.0)	19	23.75
Normal Range (1.2-20.0)	61	76.25
Lower Range (<1.2)	0	0.0
Mean	18.91 ± 16.73	
Testosterone ng/ml		
Upper Range (>10.0)	0	0.0
Normal Range (2.5-10.0)	54	67.50
Lower Range (<2.5)	26	32.50
Mean	4.0 ± 2.06	

Table 4 displays the proportion of some risk factors for male infertility. Out of the 90 patients in the study population, 24 (26.67%) were positive for ASA. Seventeen patients (18.89) had varicocoeles, 2 (2.22%) had testicular torsion while 2 (2.22%) patients had a history of mumps in childhood.

TABLE 4
Some Risk Factors for Male Factor Infertility in Port Harcourt

Characteristic	Frequency (n=90)	Percentage (%)
Anti-Sperm Antibody (ASA)		
Positive	24	26.67
Negative	66	73.33
Varicocele		
Yes	17	18.89
No	73	81.11
Torsion		
Yes	2	2.22
No	88	97.78
Mumps		
Yes	2	2.22
No	88	97.78
Sexually Transmitted Diseases		
Yes	54	60.00
No	36	40.00

In Table 5, a chi-square test of significance was performed to examine the relationship between the presence of ASAs and some risk factors for male infertility. There was a statistical difference between the presence of ASAs and age which greater . 40 years, a previous history of sexually transmitted infection/disease ($X2(1) = 5.33$, p-value = 0.02), primary infertility ($X2(1) = 16.33$, p-value = 0.001) and sperm motility < 40% ($X2(1) = 21.43$, p-value = 0.001).

Hormonal abnormalities did not play a role in ASA formation. This is also illustrated in Table 5 Furthermore, there was no significant relationship between the presence of ASAs and sperm count (X2(1) = 0.86, p-value = 0.355).

TABLE 5
Relationship between presence of ASAs and some male risk factors for infertility

Characteristic	Positive Anti-Sperm Anti-bodies		Chi-Square (X^2)	p-value
	Frequency	Percentage (%)		
Age group				
≥40	20	83.33		
<40	4	16.67	21.33	0.001*
Total	24	100.0		
Previous STD				
Yes	16	66.67		
No	8	33.33	5.33	0.02*
Total	24	100.0		
Infertility type				
Primary	19	79.17		
Secondary	5	20.83	16.33	0.001*
Total	24	100.0		
Total motility				
≤40	18	85.71		
>40	3	14.29	21.43	0.001*
Total	21	100.0		
Sperm count				
Abnormal (<20)	12	57.14		
Normal (≥20)	9	42.86	0.86	0.355
Total	21	100.0		
Testosterone				
Abnormal range	6	27.27		
Normal range	16	72.73	9.09	0.003*
Total	22	100.0		
FSH				
Abnormal range	2	9.09		
Normal range	20	90.91	29.45	0.001*
Total	22	100.0		
Prolactin				
Abnormal range	4	18.18		
Normal range	18	81.82	17.82	0.001*
Total	22	100.0		
LH				
Abnormal range	6	27.27		
Normal range	16	72.73	9.09	0.003*
Total	22	100.0		

**Statistically significant (p<0.05)*

DISCUSSION

There have been suggestions that the presence of ASAs may be a cause of infertility. These anti-bodies are said to impair fertility by affecting sperm motility and transport. ASAs cause sperm to agglutinate, thereby hampering their progress through the cervix into the uterus (17). ASAs hinder sperm motility by immobilising the sperm, interposing between sperm-mucus interactions or upsetting sperm transport (4). ASAs have also been said to affect fertility by also interposing between sperm-oocyte binding and sperm-egg interactions; ASAs hinder acrosome reaction and binding to the zona pellucida of the ovum without which fertilisation does not occur (18).

Anti-sperm anti-bodies are comprised of three immunoglobulin isotypes. They are IgG, IgA and IgM (13). These anti-bodies interact with the antigens on the sperm head, midpiece and /or tail. ASAs can be found in semen, seminal plasma, cervical mucus, serum and on spermatozoa and their presence can be detected using a variety of assays. Common tests used for the detection of ASAs include the direct and indirect immunobead test (IBT), the direct and indirect SpermMar tests, the agglutination tests - the tray agglutination test (TAT), the gelatin agglutination test (GAT) and the mixed agglutination reaction/mixed anti-globulin reaction (MAR).Other tests which have been employed to detect ASA include the enzyme-linked immunosorbent assay (ELISA), Flow cytometry (FCM), the micro immobilisation test (MIT), the sperm immobilisation test (SIT), the sperm-mucus Interaction (SMI), the sperm-cervical mucus contact test (SCMCT)(19).

This study utilised indirect SpermMar IgG test which detects circulating anti-bodies in serum. Serum was used because it could be stored frozen for a long period and storage does not affect the ASAs adversely (9). This was important as patient samples were collected over a period. The SpermMar IgG test was also used because of cost considerations in doing both an IgA and IgG test. Literature review shows that though IgA seems to be more relevant in a clinical sense than IgG. IgG hardly occurs without IgA (20-22). Therefore, IgG testing is adequate as a routine screening method.

From this study, it was observed that 26.67% of the patients tested positive for ASA screening, showing a sero-prevalence of ASAs in infertile males in Port Harcourt to be 26.67%. This result is slightly higher than that obtained in the study done in Lagos which obtained 22.7% prevalence in infertile men, using the ELISA technique (13). However, the study done by Ekwere *et al* (14), obtained a prevalence of 44%, using a combination of gel agglutination technique (GAT) and slide agglutination technique (SAT). The later finding is about two times higher than that obtained in this study and the one in Lagos. The prevalence obtained in Port Harcourt and Lagos is similar and this could be due to the fact that they are both industrial and commercial cities with similar demographics. Also the fact that different techniques were used in these tests may account for the observed different prevalence rates reported by Ekwere (14).

In other countries, the prevalence of ASAs ranged between 6 - 10% in infertile males (9, 15, 23, 24), which is much lower than that obtained. The reason for this remarkable difference is unknown. Ekwere *et al* had suggested that the high prevalence observed in their study could be due to the high prevalence of sexually transmitted infections/ diseases in their area of study (14). He reported that out of 35 patients, 22 had a sexually transmitted infection/disease and out of these 22, 13 showed the presence of circulating ASAs.

It was observed in this study that 16 out of the 24 patients who tested positive to ASA had a history of STIs. It appears that infections with STDs may account for the high prevalence recorded in this study. It must however be noted that ASAs detected in serum / cervix may not be 100% specific. Molecular mimicry may be responsible for false positive results. Molecular mimicry occurs when an infectious agent has an antigenic determinant that is similar to a self antigen (5). A study by Prabha and Vander (25), reported that a fluorescein Isothiocyanate (FITC) labeled sperm immobilisation Factor (SIF) was isolated from *Staphylococcus aureus* bound to both spermatozoa and some bacteria (both gram positive and negative).There is a possibility that some STDs especially those of bacterial origin such as *Neisseria gonorrhoea, Chlamydia trachomatis*, *Trichomonasvaginalis, Streptococcus faecalis* could have common anti-genicity with spermatozoa (25).

Hossain *et al* (9) suggested that the prevalence of ASAs increased with age. They suggested that the age factor may contribute to ASA production and that different age groups may not be equally vulnerable to immunological imbalances. This observation is in line with the results of this study, where 83.33% of those who were positive for ASAs were within the age range of 40 years and above.
The present study found that there was a significant association between the presence of ASAs type of infertility (primary or secondary) and total sperm motility. It was found that 85.71% of those affected with ASAs had total sperm motility less than 40%. Other authors had observed that ASAs reduce sperm motility by agglutination and immobilisation (4, 12) .WHO (26) also reported that ASA positive semen samples had a significant abnormal sperm count, low motility and morphology (26).

However, the hormonal levels of testosterone, FSH, prolactin and LH of the patients studied largely fell within normal ranges. This observation could show that there was no association between presence of ASAs and hormonal abnormalities.

Ekwere reported that 29% and 21% of infertile male patients had low testosterone and prolactinaemia respectively (14). Among these 18% and 13%, respectively, had ASAs. The results of this present study showed that out of 22 cases that were positive for ASA, 27.27% had low testosterone, 9.09% had high levels of FSH, 18.18% had prolactinaemia and 27.27% had high levels of LH. These results which are similar to those obtained by Ekwere suggest that the presence of ASA in serum is not significant in relation to hormonal abnormalities (14).

CONCLUSION

Anti-sperm anti-bodies are fairly common in Port Harcourt and immunological factors may contribute to infertility in our patients. Probable risk factors for ASA formation include old age, primary infertility, previous infection with

STIs and poor sperm motility. Hormonal abnormalities may not affect the production of ASAs. When this study is added to literature, anti-sperm anti-bodies would have been found in three cities in Nigeria (Lagos, Calabar and Port Harcourt). Therefore, it is recommended that ASA screening be introduced as one of the infertility tests to be carried out in assessment of the infertile males in Nigeria. Efforts to prevent exposure to sexually transmitted infections and late marriages in our locality will reduce prevalence of anti-sperm anti-bodies. Physicians should consider the possibility of immunological infertility when faced the dilemma of unexplained infertility. There is the need for further studies to determine the impact of these anti-bodies in the semen, as well as the cervical canal and sera of their spouses. The concept of molecular mimicry needs further studies especially with the high rate of sexually transmitted infections in our subjects.

REFERENCES

1. Purvis, K. and Christiansen, E. Male infertility: Current concepts. *Ann. Med.* 1992; **24**: 259-272
2. Otubu, J.A., Sagay, A.S. and Dauda, S. Hysterosalpingography, laparoscopy, and hysteroscopy in the assessment of the infertile Nigerian female. *East. Afr. Med. J.* 1990; **67**:370-372.
3. Abarikwu, S.O. Causes and risk factors for male-factor infertility in Nigeria: a review. *Afr. J. Reprod. Health.* 2013; **17**:150-166
4. Nagaria, T., Patra, P.K., Sahu Jai, P. Evaluation of serum anti-sperm anti-bodies in infertility. *J. Obstet. Gynaecol. India.* 2011; **61**: 307-316
5. Bauman, R.W. Microbiology: with diseases by taxonomy, 2nd edition. San Francisco, USA: Pearson Benjamin Cummings. 2007. P.521
6. Skau, P.A. and Folstad, I. Does immunity regulate ejaculate quality and fertility in humans. *Behav. Ecol.* 2005; 16: 410-416
7. Azizi, G.R., Namaki, S., Mirshafiey, A. and Hamid, M.K. HLA antigens and anti-perm antibody production in Iranian vasectomized men. *J. Biomed. Res.* 2015; **29**: 87-90
8. Deepali, T., Vander, H. and Vijay, P. Association of anti-sperm anti-bodies with bacterial infection: An insight to infertility. *Androl. Gynecol. Curr. Res*. 2014; **2**:1-6
9. Hossain, A., Islam, N., Aryal, S. and Madanes, A. The prevalence of circulating anti-sperm antibody (ASA) in infertile population representing of all etiologies. *Mid. East. Fertil. Soc. J.* 2007; **12**:27-30
10. Hameed, N., Ahmaed, T.A., Sultana, N., Khan, D.A., Khan, N.A. and Hassan, W. The role of the anti-sperm auto anti-bodies in the management of patients with primary infertility. *J. Pak. Med. Assoc.* 1995; **45**: 203-205
11. Imtiaz, F., Alam, N., Khatoon, F. and Mahmood, A. Anti-sperms anti-bodies in infertile males attending a Tertiary care hospital in Karachi. *Pak. J. Med. Sci.* 2012; **28**: 171-174
12. Shaya RE and Al-Dabbagh NY. Detection of anti-sperm anti-bodies in relation to inhibin B in infertile men. *Raf. J. Sci.* 2013; **24**: 1-16
13. Adejumo, B.I., Oke, A.A., Emokpae, M.A., Oyeleke, A.A., Erhabor, T.A., Agba, M.I. and Oni, A. A study of anti-sperm anti-bodies among infertile subjects investigated for infertility in Lagos, Nigeria. *Ann. Biomed. Sci.* 2014; **13**:29-35.
14. Ekwere, P.D. Immunological infertility among Nigerian men: incidence of circulating anti-sperm auto-anti-bodies and some clinical observations: a preliminary report. *Br. J. Urol.* 1995; **76**:366-370.
15. Yasin, A.L., Yasin, A.L. and Basha, W.S. The epidemiology of anti-sperm anti-bodies among couples with unexplained infertility North West bank, Palestine. *J. Clin. Diagn. Res*. 2016;**10**:QC01-3.
16. Moghissi, K.S., Sacco, A.G. and Borin, K. Immunologic infertility. I. Cervical mucus anti-bodies and post coital test. *Am. J. Obstet. Gynecol*. 1980; **136**: 941-950
17. Razzak, A.H. and Wais, S.A. The infertile couple: a cohort study in Duhok, Iraq. *East. Mediterr. Health. J*. 2002; **8**:234-238.
18. Karimi, F., Khazavi, S. and Alaedini, F. Serum anti-sperm anti-bodies in fertile and infertile individuals. *Iran. J. Med. Sci.* 2008; **33**: 88-93
19. Hjort, T. Anti-sperm anti-bodies: Anti-sperm anti-bodies and infertility: an unsolvable question. *Hum. Rep.* 1999;**14**:2423-2426.
20. Rumke, P. The origin of immunoglobulin in semen. *Clin. Exp. Immunol.* 1976; **17**: 287-297
21. Jager, S., Kremer, J., Kuiken, J. and Van Slochteren-Draaisma, T. Immunoglobulin class of anti-spermatozoal anti-bodies from infertile men and inhibition of in vitro sperm penetration into cervical mucus. *Int. J. Androl.* 1980; **3**: 1-14
22. Kay, D.J. and Boettcher, B. Comparison of the SpermMar test with currently accepted procedures for detecting human sperm anti-bodies. *Reprod. Fertil. Dev.* 1992; **4**: 175-181
23. Pattinson, H.A. and Mortimer, D. Prevalence of sperm surface anti-bodies in the male partners of infertile couples as determined by immunobead screening. *Fertil. Steril.* 1987; **48**: 466-469
24. Hadinedoushan, H. and Ghafourzadeh. .A survey of anti-sperm anti-bodies in infertile couples. *Iran. J. Reprod. Med*. 2007; **5**: 39-40
25. Prabha, V. and Vander, H. Molecular similarities between spermatozoa and bacteria: A fluorescent microscopy study. Current Microscopy Contributions to Advances in science and Technology, Mendez-Vilas, Formatex Research Center, Spain. 2012; 112-118 http://www.academia.edu/6634828/Current_microscopy_contributions_to_advances_in_science_and_technology ....accessed November 29,2016
26. World Health Organization and Department of Reproductive Health and Research (eds) WHO laboratory manual for the examination and processing of human semen. 5th Edition, WHO Press; 2010. www.who.int/reproductivehealth/publications/infertility/9789241547789/en...accessed on November 29, 2016.