

The Epidemiology of Anti-Sperm Antibodies Among Couples with Unexplained Infertility in North West Bank, Palestine

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ABSTRACT

Introduction: Anti sperm antibodies (ASA) can present in serum and semen and they may lead to impair the sperms function leading to infertility. The precise mechanism of generation of these antibodies is yet to be discovered.

Aim: This study was performed to determine the prevalence of anti-sperm antibodies (ASA) in patients with unexplained infertility. The study was initiated also to explore the possible factors that may associate with ASA formation and how ASA status is associated with pregnancy rates after going with in vitro fertilization – intracytoplasmic sperm injection (IVF-ICSI).

Materials and Methods: A cross-sectional study was conducted on 42 normal infertile couples consulting Razan Medical Center for Infertility & I.V.F. in Nablus, Palestine, from December 2012 – March 2013. Serum levels of immunoglobulins G (IgG) ASA were measured in participants (males and females) using enzyme-linked immunosorbent assay (ELISA). In addition, participants also filled a questionnaire about the presence of previous varicocele repair, inguinal hernia repair, orchitis, testicular trauma and vasectomy reversal among males and severe coitus bleeding and coitus during menses or puerperium

among females. Couples were also asked about previous IVF-ICSI procedures and the outcome of the procedure in terms of either they got pregnant or not. Data was analysed using SPSS software.

Results: The prevalence of ASA was 14.3% (6/42) among all couples, 9.5% (4/42) among males and 4.8% (2/42) among females. There was no significant relationship between previous varicocele repair, previous inguinal hernia repair, or orchitis and formation of ASA (p value =0.64, 0.56, and 0.26 respectively). Previous trauma, vasovasostomy, severe coitus bleeding and coitus during menses or puerperium were not observed in any of the study sample. ASA did not seem to affect the outcome of IVF-ICSI (p-value =0.54).

Conclusion: Prevalence of ASA in infertile couples in the north part of Palestine is similar to that obtained worldwide. ASA formation does not relate to any of the studied risk factors and does not seem to associate with pregnancy rate after IVF-ICSI. We recommend further studies using a larger sample size and including all parts of Palestine in order to generalize the obtained results.

Keywords: Immune response, Intracytoplasmic sperm injection, Pregnancy, Invitro fertilization, Prevalence

INTRODUCTION

Infertility is defined as the inability of couples to achieve pregnancy after 12 months of unprotected sexual intercourse. This condition affects 10-15% of couples worldwide [1]. In Palestine, and according to Palestinian Central Bureau of Statistics (PCBS), 8.4% of couples aged 15-49 years reported infertility. Overall factors responsible for infertility, male factors comprise 30-40% in the male, female factors 40-55%, and combined factors comprise nearly 10%. In approximately 10% cases the cause of infertility remains unexplained, and the couple is designated as normal infertile couple [2].

Several studies in different areas showed wide variety in the prevalence of ASA for example a study conducted at USA [3] reported an ASA prevalence of 5.7%. Data on the frequency of ASA in infertile men have shown high variability (from 7% to 44%), depending on the method applied [4-7]. In contrast, researches have shown that the frequency of circulating ASA ranges from 1.3% to 7.3% in serum of infertile women [7-9].

Recently, there has been a lot of discussion about the role that the immune system plays in conception. The concept that an adverse immune response against reproductive system can result in infertility is now more recognized than before [10-12]. ASA is an immunological markers that is considered in the evaluation of immunological in fertility [13-15]. Serum was positive for ASA in about 10% of men and 5% of women with unexplained infertility [16]. However, infertility managements around the world exclude ASA screening among infertility patient. This is due to the fact that there is conflicting information on the significance of ASA in the aetiology of infertility [17-19]. Therefore, there is a scarcity of data about ASA in the literature we have [11, 17].

In Palestine, ASA testing is sometimes ordered by some physicians when they are evaluating infertile patients, however there is no any local studies describing the prevalence of ASA, therefore, we need a baseline study in Palestine to find prevalence and risk factors of ASA to determine the size and burden of the problem.

AIM

This study was initiated to investigate the prevalence of ASA among patient with unexplained infertility in North West Bank (NWB). In addition to our main objective, the study was initiated also to explore possible factors that are associated with ASA formation in both sexes and how ASA status is associated with pregnancy rates after going with IVF-ICSI.

MATERIALS AND METHODS

In this cross-sectional study, 42 couples with unexplained infertility who were attending Razan Medical Center for Infertility & IVF in Nablus city between December 2012 - March 2013 were included. This study was approved by the Ethics Committee of An-Najah National University and a signed consent was obtained from each participant. The study included couples from North West Bank who were diagnosed with unexplained infertility and did not use any form of contraception for more than one year. According to PCBS, the number of couples in North West Bank (NWB) which includes Nablus, Jenin and Tulkarm cites is nearly 170000; 8% of them are infertile [20]. Then by using automated sample size calculator (Creative Research Systems, 2012) [21] with a 95% confidence level, the calculated sample size was 50 couples at most, and since not all of them will present to Razan center, a sample size of 40-50 was considered appropriate.

After obtaining a signed consent from couples who met the criteria for selection, couples were asked to fill a simple questionnaire about demographic data and potential risk factors for development of ASA, an it includes previous varicocele repair, testicular trauma, testicular infection, inguinal hernia repair, vasectomy reversal, coitus bleeding, and coitus during menstrual cycle or puerperium and outcome of previous IVF-ICSI attempts.

Under aseptic conditions, venous blood of about five ml was withdrawn from each study participants. Then 5 minutes of centrifugation at 3000 rpm was used to separate Serum from the whole blood. Then serum samples were kept at -20°C in sterile microtubes until the time of serological testing. ASA was tested by enzyme-linked immunosorbent assay (ELISA) test kit (DRG Instruments GmbH, Germany. EIA-1826); following the manufacturer's instruction.

STATISTICAL ANALYSIS

The collected data was analysed using Statistical Package for the Social Sciences (SPSS) version 20. Descriptive analysis including the mean, standard deviation, and frequency distribution and analytical methods were carried out. A chi-square test was performed to find the significance of association between ASA formation and the possible risk factors mentioned above and to determine the effect association between ASA status on IVF-ICSI outcome.

RESULTS

Forty two couples (42 husbands + 42 wives = total 84 participants) who were denoted as unexplained infertile couples were tested for the presence of ASA in their sera. The duration of marriage ranged from 2 years to 21 years with an average of 6 years. The mean age of husbands was 34 years with a range of 22-46 years, while that of wives was 28 years with a range of 20-40 years.

The prevalence of ASA was 14.3% (6/42) among all couples, 9.5% (4/42) among males and 4.8% (2/42) among females. Positive ASA results were distributed through the three cities in NWB as shown in [Table/Fig-1,2].

According to our study there was no association between varicocele repair, inguinal hernia repair or orchitis and ASA development as shown in [Table/Fig-3].

However, none of males either ASA positive or negative reported a history of previous testicular trauma or vasectomy reversal. In the same time neither ASA positive nor ASA negative females had a history of severe bleeding during coitus or sexual intercourse during menses or puerperium.

In order to overcome the problem of infertility, 22 couples managed with IVF-ICSI, and it was found that no relation between ASA status and the successfulness of IVF-ICSI exists ($p=0.54$). It is worth to mention that neither husband age nor wife age was statistically a significant factor regarding outcome of IVF-ICSI ($p=0.78$ for husband age and 0.53 for wife age). This is shown in [Table/Fig-4].

ASA status	Nablus	Jenin	Tulkarm	Total
ASA positive	1 (6%)	3 (20%)	2 (18%)	6
ASA negative	15 (94%)	12 (80%)	9 (82%)	36
Total	16	15	11	42

[Table/Fig-1]: Distribution of cases according to place of living and ASA status among couples.
Abbreviations: ASA, antisperm antibodies.

ASA status	Nablus		Jenin		Tulkarm		Total	
	Male	Female	Male	Female	Male	Female	Male	Female
ASA positive	1 (6%)	0 (0%)	1 (6.6%)	2 (13%)	2 (18%)	0(0%)	4	2
ASA negative	15 (94%)	16 (100%)	14 (93.4%)	13(87%)	9 (82%)	11 (100%)	38	40
Total	16	16	15	15	11	11	42	42

[Table/Fig-2]: Distribution of cases according to place of living and ASA status among individuals.
Abbreviations: ASA, antisperm antibodies.

Variables	Total sample (Total: 42)	ASA positive Group (Total: 4)	ASA negative Group (Total: 38)	p-value*
History of varicocele	17(41%)	1(25%)	16(42%)	0.64
Inguinal hernia repair	12(28.5%)	2(50%)	10(26%)	0.56
Previous orchitis	3(7%)	1(25%)	2(5%)	0.26

[Table/Fig-3]: Relationship between ASA status and male risk factors
Abbreviations: ASA, antisperm antibodies p-value< 0.05 was set significant.
*Fisher exact test.

ASA status	Successful IVF-ICSI	Failed IVF-ICSI	Total
ASA positive couples	6 (33%)	0 (0%)	6
ASA negative couples	12 (67%)	4 (100%)	16
Total	18	4	22

[Table/Fig-4]: Distribution of cases according to couple ASA status and IVF-ICSI outcome.
Abbreviations: ASA, antisperm antibodies; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

DISCUSSION

Immunoinfertility is one of the major causes of infertility in humans which entails production of specific auto antibodies against sperms [9]. However, the relevance of ASA in the diagnosis and treatment of infertility is still controversial [22]. The current study will try to help to give an answer to this controversy.

In this study, overall, 7.1% of all participants were ASA positive which is slightly higher than a similar study conducted at USA [3] and reported an ASA prevalence of 5.7%. The prevalence of ASA reached 9.5% among males. Data on the frequency of ASA in infertile men have shown high variability (from 7% to 44%), depending on the method applied [4-7]. And it reached 4.8% among females with unexplained infertility, Other researchers reported the frequency of circulating ASA from 1.3% to 7.3% in serum of infertile women [7-9]. This variability may be also related to the tool used to detect ASA and sample size.

No significant association was noted between the presence of ASA and previous varicocele repair, previous inguinal hernia repair or orchitis. This goes in parallel with the fact that despite the long list of risk factors associated with ASA development, most cases are idiopathic and all infertile males are at risk [23].

It is possible that the identification of a statistical association between ASA and the presence of risk factors was not found due to the small sample size. However, significant associations could be found if larger sample size was taken. For example, a study conducted on 230 couples in Nugegoda showed significant association between varicocelectomy and inguinal herniorrhaphy on one hand and the ASA status on the other hand [24]. These surgeries would breach the blood testis barrier and facilitate formation of ASA.

Genitourinary tract inflammation and infection may potentially lead to genital tract disruption and ASA formation. Acute epididymo-orchitis leads to sperm autoimmunity in 27% of a series of 32 men [25]. Experimental orchitis in rabbits leads to ASA formation [26]. In the current study, 30% who suffered previous attack of orchitis develop ASA.

Vasectomy reversal and testicular trauma were not observed in any of the study sample, this may be due to the fact that vasectomy is a very rare operation in Palestine since sterilization is prohibited in Islam, meanwhile, another study showed that after vasectomy, approximately 50% of men produced ASA [27]. Also a significant increase in the postoperative incidence of the antibodies was seen in the men who achieved patency after vasectomy reversal but not in those men for whom no sperm were seen in the ejaculate [28], this is due to sperm extravasation which occurs during and after the surgery and disruption of the blood testes barrier. On the other side testicles are infrequently prone to trauma due to their location between thighs. Taking larger sample size will at least solve the problem of testicular trauma.

Female genital tract mucosal layer disruption either due to mechanical or chemical cause will lead to exposure of the sperm antigens to the immune system which will result in production of

ASA [24]. So that, it is presumed that harsh sexual intercourse with heavy bleeding or coitus during times of uterine mucosal disruption for example during menses and puerperium, will bring huge amount of sperms in direct contact with circulation and so definite immune response will take place.

Despite that, this is not the scenario, and unlike the experience in homosexual men, sexual practices have not been demonstrated to be a risk factor in women [29]. In addition, it is not clear why most women who are exposed to sperm do not make an immune response [23].

Considering cultural, social and religious limitations, the number of couples who were willing to answer the questions and talk frankly about their personal sexual life was nil in the present study, and so severe coitus bleeding or coitus during menses or puerperium cannot be evaluated as risk factors for ASA formation. None of ASA positive couples and only 25% (4/16) of ASA negative couples experienced IVF-ICSI failure. This means that ASA may not be a major factor interfering with IVF-ICSI successfulness. Worldwide, several studies have investigated the relationship between ASA and pregnancy rate after IVF and ICSI, but the results have been inconsistent. A recent systematic review and meta-analysis indicated that ASA are not related to pregnancy rates after IVF-ICSI, suggesting this procedure as a viable options for infertile couples with ASA [30]. Another previous study demonstrates that ASA testing has low sensitivity in predicting low or no fertilization and does not appear to be cost-effective when selectively ordered as part of an IVF-ICSI workup [31].

Several IVF centers claim that testing for ASA is "of academic interest only" [32], as the results have no relationship with pregnancy rate following IVF and that the actual quantification of antibodies is of less importance than other parameters such as sperm concentration and motility [33].

LIMITATION

However, knowing the limitation of our study of a small sample size, we recommend larger multi-centers studies which are adequately powered to draw a conclusion on this conflicting issue. In Addition, only serum ASA titers were measured. ASA in cervicovaginal secretions and sperm bound antibodies could have also been measured, so we recommend future studies to be based on them.

CONCLUSION

In conclusion, the prevalence of ASA in NWB is similar to that obtained worldwide when ELISA is used to detect ASA. No association was found between varicocele repair, inguinal hernia repair and orchitis in one hand, and the increased risk for ASA formation on the other hand, and all infertile men seem to be at risk for being ASA positive. In addition, ASA do not appear to interfere with the outcome of IVF-ICSI.

In Palestine, this study is very useful for establishment of a baseline data about the prevalence and risk factors of ASA to determine the size and burden of the problem. This will help infertility and IVF centers to adopt best strategies in diagnosis and management of unexplained infertility. To our knowledge, our study will be the first to do that. However, further studies with larger number of patients including more details regarding the clinical variables and cover larger areas and multiple centers should be carried out to confirm our findings. We recommend that ASA assessment should be considered as an essential part of infertility management. Only IVF-ICSI seems able to overcome the problem.

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