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Evaluation of Antisperm antibodies in the blood of unmarried women: A case study

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Abstract
This article aims at studying, in more details, the possible causes and interpretations behind the development of antisperm antibodies in virgins. This descriptive case study included 35-years old single women with positive ASA. The mean serum antisperm antibody concentrations is (62.1 IU/ml), it is considered as positive titer. Complete blood count is normal, except for slight increase in WBC count and percentage of basophils, monocytes and lymphocytes. This study recorded high concentrations of serum total IgG and IgM levels (1870 IU/ml and 255 IU/ml respectively). The UTI was confirmed by counting total bacterial concentration (167220 CFU/ml) in the urine. There is two suggested mechanisms to explain ASA in virgins: (1) antigen cross-reactivity between sperm and bacterial antigens to which antibodies can react; () 2induction of the immune system by antigens of sperm ingested into the gastrointestinal tract with contaminated food and drink.

Keywords: Antisperm, antibodies, virgins, infertility, case-study

Introduction

It is well-known that study of antisperm antibodies ASA etiology is very important in the diagnosis and treatment of infertility. A number of hypotheses have been suggested to explain the etiology of antisperm antibodies in both genders. In particular, several controversial studies had been conducted to investigate the occurrence and types of antisperm antibodies in women [1]. Sperm is considered as a highly diverse antigenic cell, it contains antigens that can be modified during processes of maturation and ejaculation, e.g. the binding of soluble fibronectin to the tail of sperm. During the early years of the 20th century, researchers had shown that spermatozoal and seminal plasma antigenic components can stimulate both specific autoimmunity and isoimmunity [2]. For women exposed to sexual activity, antisperm antibodies may be developed in their body after spermatozoal discharge into the reproductive tract with a immunologically settled epithelial tissue, the peritoneal cavity, or the digestive tract [3]. Exposure to sperm after oral or anal intercourse has been associated with the formation of antisperm antibodies in both women and homosexual men [4]; Wolff and Schill (1985) investigated ASA in men involved in forbidden homosexual intercourse, they recorded a high incidence of antisperm antibodies in the sera of homosexual men, they explained that high incidence by inducing immune system with antigens from spermatozoal cells through passive anal intercourse [5].

It was found that cervical epithelial mucus is an essential element of immune system with high capacity in responding to microbial infectious agents, foreign antigens and, occasionally spermatozoal antigens. Plasma cells of the sub-epithelial layer of ovarian tubes, uterus and vagina, have the ability to produce immunoglobulin class A [6-7]. Like other mucosal surfaces, the secretions and tissues of the female genital tract contain antibodies, antibody-producing cells (plasma cells) in addition to other lymphatic cells, macrophages, and helper T lymphocytes [8]. Secretory IgA is the most abundant immunoglobulin in the genital tract secretions, especially in cervix, uterus and uterine tubes. Other types of immunoglobulins like IgG and may be IgM are also present. The ratio of IgA: IgG in cervical is much higher in mucus than that in serum. [6]. The identification of Ig subclasses of cervical mucus antibodies showed that about 70% of the IgA was IgA_1, whereas IgG4 and IgG3 were the major IgG subclasses present [7- 9]. It has been suggested that antisperm antibodies may form in women because of a decrease of one or more immunosuppressive factors in her husband's seminal fluid as sperm cells may possess protective mechanisms that inhibit immune reactions, but there is no convincing evidence to aid this hypothesis [10-11].

Many authors mentioned that the female have the ability to potentially form anti-idiotype antibodies that are directed against the male husband’s antibodies specific for internal sperm structures, as well as to antibodies specific for antigenic structures of sperm cells [12]. The hypothesis of idiotype antibodies is widely supported by the studies of several researchers [13].

Although antisperm antibodies in married women and adult males are well studied, no adequate studies were conducted to investigate the possible presence of antisperm antibodies in the bodies of unmarried women that have not exposed sexual activity (virgins). Many authors did not found any levels of ASA in the serum of virgin women [7], others have recorded detectable concentrations of ASA in the serum of unmarried women that did not have any sexual activity [14].

This article is a case study about the occurrence of antisperm

antibodies in the serum of a virgin women. It aims at studying, in more details, the possible causes and interpretations behind the development of antisperm antibodies in virgins.

2. Methodology

2.1 Patients Description

The patient is 35-years old single women, has been working as a technician in the hospital laboratory for about 9 years. As a result to her usual and routine work in the laboratory, she daily deals with clinical samples like blood, serum, semen and sputum. She lives in rural area, with a sufficient monthly income, and a stable family conditions. She doesn't smoke, and has a borderline normal Body Mass Index (BMI = 24.2, based on: 165 cm height and 66 kg weight). She has been selected as a volunteer in this research because of a positive ASA in her serum after investigating 56 volunteer single women with a negative serum ASA. Assessment and all laboratory investigations were achieved between 1st to 15th December 2018.

2.2 Clinical History

The patients has a regular menstrual period, no conditions of bleeding previously took place, ultra sound imaging (performed by a specialized radiologist) do not reveals any signs of polycystic texture or tumors (figure 1). She does not suffer from hypertension or diabetes mellitus. Digestive cramps, irritable bowel syndrome or colitis may usually occurs. Skin and respiratory allergies frequently develop as a result to different irritant. Recurrent urinary tract infection is the main infectious disease that becomes the first health problems for the patient. She is not exposed to any surgical or diagnostic procedure during her life. No drug is taken at the time of assessment, except for administrating esomeprazole (20 mg once a day) for irritable bowel syndrome and as a prophylactic treatment for peptic ulcer

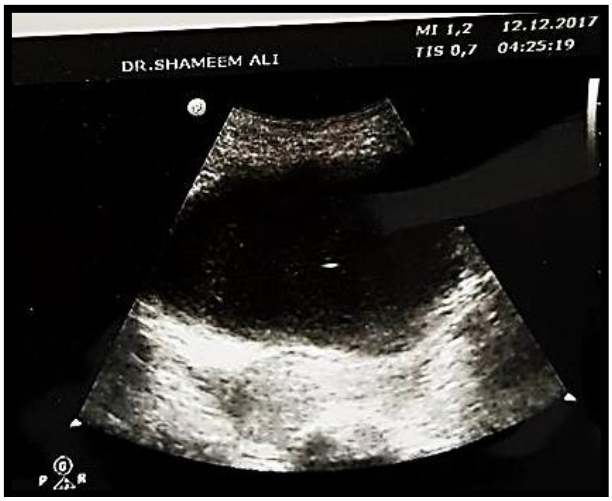

Fig 1: Ultrasound image for the ovarian and the uterine tissue of the patient indicating normal texture without any polycystic or tumor finding.

2.3 Laboratory Tests

2.3 1. Investigation of Serum ASA

The concentration of serum ASA was estimated by using Enzyme-Linked Immunosorbent Assay (ELISA) kits equipped by DRG International, Inc., USA. The cutoff point for serum ASA concentration was 60 IU/ml (above which the sample was considered positive as fixed on the kit instruction). To avoid unintended technical error, the test was repeated three times on different periods, the mean value was calculated [15].

2.3.2. Complete Blood Count

For counting complete blood picture, 3 ml of blood was taken by using sterile syringe and put in heparinized tube, the test was done by using HumaCount System (Germany).

2.3.3. Total Serum IgG and IgM

Radial immunodiffusion is a simple technique that is routinely used for measuring the concentrations of various soluble immunoglobulines (usually proteins) in biological fluids. It is principally derived from the work of Mancini [16].

2.3.4. Urine Culture

Urine sample was cultured on Blood and MacConkey agar. Finding of 100,000 colony-forming units per milliliter (CFU/mL) or more was a "positive" test result symbolizing infection. The isolated bacteria were identified according to microscopically, biochemical tests and VITEK 2 system (bioMérieux) [17].

3. Results

Table (1) shows the mean (62.1 IU/ml) and standard deviation (1.2 IU/ml) serum antisperm antibody concentrations, it is considered positive depending on the kit instruction. This result was obtained after testing 56 negative subjects. According to table (2), measures of the complete blood count are normal except for WBC count and percentage of basophils, monocytes and lymphocytes, which were slightly shifted toward the high levels. Table (3) shows a high concentrations of serum total IgG and IgM levels (1870 IU/ml and 255 IU/ml respectively).

Table 1: Mean and standard deviation of serum Antisperm Antibodies

Measure	Mean ± SD	Assessment
Serum ASA (IU/ml)	62.1 + 1.2	Positive

Table 2: Values and Assessment of Complete Blood Count

Measure	Value	Assessment
RBC Count (cell\mm3)	$4.3 * 10^6$	Normal
WBC Count (cell\mm3)	9800	Borderline (High)
Hb (g/dl)	13.6	Normal
PCV (%)	37.4	Normal
Platelets Count (cell\mm3)	228500	Normal
Neutrophils (%)	55.2	Normal
Basophils (%)	0.8	Borderline (High)
Eosinophils (%)	3.6	Normal
Monocytes (%)	6.2	Borderline (High)
Lymphocytes (%)	34.2	Borderline (High)

Table 3: Values of Total Serum IgG and IgM

Measure	Value	Assessment
Total Serum IgG (IU/ml)	1870	High
Total Serum IgM (IU/ml)	255	Borderline (High)

As shown in tables (4) and (5), the UTI was confirmed by counting total bacterial concentration (167220 CFU/ml), and diagnosis of bacterial species that involve in the infection, they

include: *Escherichia coli, Klebsiella pneumonia, Staphylococcus haemolyticus, Proteus mirabilis.*

Table 4: Values of Total urine Bacterial Count

Measure	Value	Assessment
Total Bacterial Count (CFU/mL)	167220	Positive UTI

Table 5: Common Bacterial Isolates from the Patient's Urine

Isolates
Escherichia coli
Klebsiella pneumonia
Staphylococcus haemolyticus
Proteus mirabilis

4. Discussion

It is understandable to find very few data about the occurrence of ASA in virgins, one reason may return to the misconception that ASA can't be detected in virgins, the other reasons is perhaps due to social determinants which assumes that sperm never contact with virgin body except through sexual intercourse. Out of 56 single women investigated in this study, one was positive for ASA by using ELISA method, this positive case was the subject on the current case-study article. Some researchers had got results similar to the present study; perhaps the first positive in the last century case was detected by the work of Harrison (1976), he used immunofluorescent assay to investigate for ASA in 66 virgin women and found that approximately 33 % of them are positive for ASA [18], Saji *et al*. (1988) studied the presence of ASA in the sera of 3 virgin women, they found that one of them (33.33%) had a positive ASA test [19].

According to the assessment performed in this study and the information provided by previous literature, two hypotheses can be suggested to interpret the occurrence of ASA in the serum the unmarried women (virgins); the first hypothesis is built on observations that human sperm cell have antigens that show immunological cross-reactivity with certain microbial antigens and auto antigens. Common cross-antigenicity has been observed between human and mouse spermatozoa and bacteria, parasites, viruses, fungi, and allergens [20-21]; Shi *et al*. (2007) recorded that common cross-reactive antigens for immunocontraceptives can be detected between microorganisms and sperm which may induce subfertility [22]. Kennedy *et al*. (1988) concluded that cross-reactivity between sperm antibodies and autoantibodies, usually arises in autoimmune disease, suggests that of activation of polyclonal B cell, like that seen in autoimmune diseases, may occur in patients with sperm antibodies [13].

In the present study, the clinical history revealed recurrent UTI, the laboratory investigations showed that the main bacterial causes of which are: *Escherichia coli, Klebsiella pneumonia, Staphylococcus haemolyticus, Proteus mirabilis.* Antigens from these bacteria may have common cross-reactivity with sperm antigens, this hypothesis can be supported by the researched conducted by a many researchers, they detected the finding of cross-reaction between the spermatozoa antigens and many microbes by different ASA diagnosis techniques. Antigens that are common among those microbes have been found between sperm cells and each of: Klebsiella pneumonia, Escherichia coli, Streptococcal antigens, Staphylococcus aureus, Trichomonas vaginalis, Candida albicans, Ureaplasma urealyticum and Mycoplasma hominis [22-25].

The clinical history in the current study also revealed that patients suffered from the colitis and accompanying digestive cramps, Inflammatory bowel diseases (IBD) can be defined as prolonged inflammatory illness and collapsing disorder of the GIT that lead to alteration in gut histological structure and function [22-23]. This inflammatory process leads to elevated intestinal penetration and congestion in irritable bowel syndrome patients, and subsequent vaccination against pathogenic or gut antigens of normal flora, which seem to have similar antigenic locations with cells of sperm [26]. Rossato and Foresta (2004) reported that sperm may share some antigenic structures similar to structures against which the immune system is directed in patients with IBD [27], while Dimitrova *et al*. (2005) found that there is a statistically significant increase in ASA occurrence in ulcerative colitis patients comparing to the control normal fertile volunteers with no signs or symptoms of IBD at similar age [28]. In same directions, Kalaydjiev *et al*. (2007) showed increased levels of serum sperm antibody titer in patients with salmonellosis and shigellosis [29]; Dimitrova-Dikanarova *et al*. (2017) showed a linear correlation between anti-H. pylori antibodies and ASA, suggesting a role of H. pylori infection in the induction of ASA [30]. In spite of these findings, some researchers did not confirm such cross-reaction association [31].

The second hypothesis is that we present a new suggestion about the mechanism by which ASA are produced; the virgin women under study, as we mentioned in this article, has worked in the hospital laboratory for many years, she handles semen samples every day, hence it is probable that sperm are ingested into the gastrointestinal tract through different ways, such as by contaminated hands, or contaminated food and drinks. This probability can be strengthened by the fact of poor hygiene is found in all health institutions in Iraq. This suggestion may supported by the work of many investigators who observed incidence of ASA in the serum of women with oral sexual practice [4], although those researchers did not find statistically significant difference in the incidence of ASA in those women, they found a slight increase in the incidence of serum ASA; Chaco *et al*. (1991) found that approximately 56.6% of women with oral intercourse have developed serum ASA [32], Yazdi *et al*. (2009) found positive ASA cases in about 20% of women exposed oral sexual activity [33].

5. Conclusions

The development of ASA in the patients included in this case-control study article can be understood by two suggested mechanisms: (1) antigen cross-reactivity between sperm and bacterial antigens to which antibodies can react; (2) induction of the immune system by antigens of sperm ingested into the gastrointestinal tract with contaminated food and drink.

As the studies of the ASA in the virgins are very rare, it is recommended to achieve a research study that include a large number of virgins for investigating ASA to confirm our results and build a scientific generalizations. we also recommend to conduct animal studies to test the role of GIT-deposited sperm in the induction of immune system.

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7. References

1. Isojima S. Human sperm antigens corresponding to sperm-immobilizing antibodies in the sera of women with infertility of unknown cause: personal review of our recent studies. Hum Reprod. 1989; 4(6):605-12.
2. Kosanovic M. and Jankovic M. Molecular heterogeneity of gelatin-binding proteins from human seminal plasma. Asian J. Androl. 2012; 12**,**363-375.
3. Marshburn B, Kutteh WH. The role of antisperm antibodies in infertility. Fertil Steril. 1994; 61(5):799-811.
4. Chacho KJ, Hage CW, Shulman S. The relationship between female sexual practices and the development of antisperm antibodies. Fertil Steril. 1991; 56(3):461-4.
5. Wolff H, Schill WB. Antisperm antibodies in infertile and homosexual men: relationship to serologic and clinical findings. Fertil Steril. 1985; 44:673.
6. Marshburn PB, Kutteh WH. The role of antispermantibodies in infertility. Fertil Steril. 1994; 61:79-811.
7. Naz RK, Menge AC. Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility. FertilSteril. 1994; 61:1001-13.
8. Beer A, Neaves W. Antigenic Status of Semen from the Viewpoints of the Female And Male. Fertility and Sterility. 1978; 29(1):3-22.
9. Brazdova A, Vermachova M, Zidkova J, Ulcova-Gallova Z, Peltre G. Immunodominant semen proteins I: New patterns of sperm proteins related to female immune infertility. Cent. Eur. J. Biol. 2013; 8(9):813-818.
10. Olivares S, Villanueva-Diaz C, Hernandez C, Arredondo J, Vadillo-Ortega F. Identification and partial characterization of an immunoglobulin A protease associated with human sperm. Bioi Reprod. 1993; 49:162-5.
11. Witkin S. Failure of sperm -induced immunosuppression: association with antisperm antibodies in women. Am J Obstet Gynecol. 1989; 160:1166-88.
12. Clarke G. N. Etiology of sperm immunity in women. Fertil Steril. 2009; 91:639-43.
13. El-Roeiy A, Valesini G, Friberg J, Shoenfeld Y, Kennedy RC, Tincani A, *et al*. Autoantibodies and common idiotypes in men and women with sperm antibodies. Am J Obstet Gynecol. 1988; 158(3 Pt 1):596-603.
14. Dodsworth NJ, Knight V, Rohrsheim R. Why virgins attend sexually transmitted disease clinics. International Journal of STD & AIDS. 1993; 4:232-233.
15. Diagnosis-Related Group (DRG). Sperm Antibody ELISA. DRG® International, Inc., USA, 2012.
16. Mancini G, Carbonara AO, Heremans JF. Immunochemical Quantitation of Antigens by Single Radial Immunodiffusion. Immunochemistry. 1965; 2:235.
17. Wilson ML, Gaido, L. Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. Clinical Infectious Diseases. 2004; 38:1150-8.
18. Harrison RF. Immunofluorescent sperm antibodies in virgins (letter). BMJ, 1976; 2:1258.
19. Saji F1, Ohashi K, Kato M, Negoro T, Tanizawa O. Clinical evaluation of the enzyme-linked immunosorbent assay (ELISA) kit for antisperm antibodies. Fertil Steril. 1988; 50(4):644-7.
20. Nakamura S, Tsuji Y, Nakata Y, Komori S, Koyama K. Identification and characterization of a sperm peptide antigen recognized by a monoclonal antisperm autoantibody derived from vasectomized mouse. Biochem Biophys Res Commun. 1994; 205:1503-9.
21. Kurpisz abd M, Alexander NJ. Carbohydrate moieties on sperm surface: physiological relevance. Fertil Steril. 1995; 63:158-65.
22. Shi J, Yang Z, Wang M, Cheng G, Li D, Wang Y, *et al*. Screening of an Antigen Target for Immunocontraceptives from Cross-Reactive Antigens between Human Sperm and Ureaplasma urealyticum. Infection And Immunity, 2007, 2004-2011.
23. Popivanov RP, Zhukov-Verezhnikov NN, Boulanov ID. Heterogeneous antigens of E. coli similar to antigens of human spermatozoa. Bull Exp Biol Med. 1981; 9:316-317.
24. Tinneberg HR, Boettcher BA, Cheng CY. Possible cross reactivity of group A streptococcal antigens and antigens related to sperm agglutination. Biol Immunol Reprod. 1981; 5:49-53.
25. Kalaydjiev SK, Dimitrova DK, Trifonova NL, Fichorova RN, Masharova NG, Raicheva YN, *et al*. The agerelated changes in the incidence of natural anti-sperm antibodies suggest they are not auto-/isoantibodies. AJRI. 2002; 47:65-71.
26. Sartor RB. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. Gastroenterol Clin North Am. 1995; 24:475-507.
27. Rossato M, Foresta C. Antisperm Antibodies in Inflammatory Bowel Disease. Arch Intern Med. 2004; 164(20):2281-2283.
28. Dimitrova D, Kalaydjiev S, Mendizova A, Piryova E, Nakov L. Circulating antibodies to human spermatozoa in patients with ulcerative colitis. Fertil Steril. 2005; 84(5):1533-5.
29. Kalaydjiev S, Dimitrova D, Mitov I, Dikov I, Nakov L. Serum sperm antibodies after diarrhoeal diseases. Andrologia. 2007; 39:101-108.
30. Dimitrova-Dikanarova DK, Lazarov VV, Tafradjiiska-Hadjiolova R, Dimova II, Petkova NU, Krastev ZA. Association between Helicobacter pylori infection and the presence of anti-sperm antibodies. Biotechnology & Biotechnological Equipment. 2017; 31(1):1-8.
31. Eggert-Kruse W, Rohr G, Probst S, Rusu R, Hund M, Demirakca T, *et al*. Antisperm antibodies and microorganisms in genital secretions—a clinically significant relationship?. Andrologia. 1998; 30:61-71.
32. Chacho KJ, Hage CW, Shulman S. The relationship between female sexual practices and the development of antisperm antibodies. Fertil Steril. 1991; 56(3):461-4.
33. Yazdi RZ, Sene AA, Kohpaee Z, Zadehmodaress S, Hosseini S, Fallahian M. The Correlation between Sexual Practices and the Development of Antisperm Antibodies. International Journal of Fertility and Sterility. 2009; 2(4):189-192.