Tests for Sperm Antibodies

13

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Abstract

Several methods have been used to identify the presence of antisperm antibodies (ASA). The clinical significance of the results derived from ASA testing methods is widely debated. The conflicting data reported by various investigators may be due to confounding factors that include the use of different ASA testing modalities, specimen preparation, and test interpretation standards. Furthermore, sperm specimens are dynamic, undergoing maturational changes including capacitation and the acrosome reaction, which results in changing ASA epitopes as the outer acrosomal membrane and its associated proteins are lost and the antigens present on the inner acrosomal membrane become exposed. Currently, there is a consensus that results of testing for ASA in seminal fluid using the mixed antiglobulin reaction and immunobead tests can be significantly associated with unexplained infertility. On the other hand, testing of seminal plasma or serum was found to be less relevant for fertility assessments.

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13.1 Introduction

The sperm antigenicity was first identified in the nineteenth century following sperm injection into a foreign species. Thereafter, it was reported that sperm can be also antigenic when injected into the same species [28]. The coating of spermatozoa with antisperm antibodies (ASA) can significantly interfere with fertility. Possible mechanisms include immobilization of the spermatozoa, impaired cervical mucus penetration, inhibition of capacitation, and disturbance in sperm-ovum interaction. Furthermore, ASA have been associated with the secretion of cytokines which impair sperm function and can eventually lead to cell death [32]. Although ASA can be detected in serum, they were not reported to impair fertility unless present within the reproductive tract or are detectable on living spermatozoa [11].

Several methods have been described for the detection of ASA. They include the tube slide agglutination test (TSAT), gelatin agglutination test (GAT), sperm immobilization test (SIT), immunobead test (IBT), and mixed antiglobulin reaction (MAR) test using sensitized erythrocytes. Despite the variety of available testing methods, the World Health Organization (WHO) Special Program of Research Development and Training in Human Reproduction has recommends only the MAR test or the IBT for the assessment of human semen [37].

There is considerable disagreement between the different testing methodologies and their clinical significance in relation to infertility. Existing data do not support the widespread use of immune testing in clinical practice. Additionally, ASA treatment options do not have confirmed benefits and may even prove to have negative effects [23]. It was previously reported that the routine use of ASA testing can have some cost saving advantage relative to the expenses encountered during in vitro fertilization (IVF) cycles [5]. Detection of ASA was considered an indication for intracytoplasmic sperm injection (ICSI) versus routine IVF since it has been shown to yield higher fertilization rates [8]. Nevertheless, it was not accepted both routine IVF and ICSI are of equal benefit in cases diagnosed with ASA.

13.2 Sites of Different Antisperm Antibody Classes

Antisperm antibodies IgM have been detected in the circulation of men; however, no traces of the IgM molecules were detected in the male genital tract. Therefore, testing for the IgM class does not appear to be of value in the context of male fertility evaluation [35]. On the other hand, 1 % of the serum IgG has been documented in the male genital tract. The presence of seminal IgG could be the result of transudation from circulation or it could be due to local antibody production [17]. As regards IgA class in human semen, it appears to be the result of local production since seminal plasma IgA is of the secretory IgA type [38].

13.3 Testing Methods

Several tests have been developed to detect and quantitate ASA, which may be categorized into groups based on the antigen source: (a) live sperm assays such as macroagglutination, microagglutination, cytotoxicity, or sperm/cervical mucus interaction tests; and (b) fixed sperm assays such as immunofluorescence, enzyme-linked immunoassays and radioimmunoassay, and mixed antiglobulin tests [4]. Currently, the mixed antiglobulin reaction test and the immunobead test are the only tests which are routinely performed by diagnostic laboratories [37].

13.3.1 Macro/Microagglutination and Immobilization

A macroscopic approach has been described to identify the presence of ASA in serum. The gelatin agglutination test (GAT) is conducted by suspending the semen from a donor known not to have ASA with the complement-inactivated serum of the suspected subfertile patient in a gelatin mix. Sperm agglutinates at the bottom of the gelatin mix can be interpreted as positive [26]. The GAT test is known to reveal false-positive results due to the presence of debris in seminal plasma. Therefore, it no longer plays a role in the diagnosis of immunological infertility especially that the clinical relevance of ASA in serum is now hugely debated. A similar test, the tube slide agglutination test (TSAT), is performed by mixing donor semen with complement-inactivated patient serum followed by detection of the sperm agglutination using a microscopic drop [16]. The TSAT is also no longer recommended as a testing modality.

The sperm immobilization test (SIT) procedure resembles the TSAT, but smaller volumes of rabbit or guinea pig serum are added as a source of complement. During microscopic assessment, the number of motile sperm is determined and the test is considered positive if more than half of counted sperm are found to be nonmotile [21]. In addition to the disadvantages noted above for the GAT and the TSAT, SIT lacks the ability to detect IgA since fixation of complement and initiation of the cascade sequence is only possible for antibodies of the IgG and IgM classes [6].

13.3.2 Tests for Cervical Mucus

The presence of ASA in cervical mucus can be assessed by using in vivo or in vitro sperm-mucus interaction tests. The in vivo postcoital test (PCT) is conducted by sampling the cervical mucus several hours after intercourse and examining it for the presence of spermatozoa. The presence of less than ten sperm/HPF could be interpreted as a poor result. Most importantly, the presence of a distinctive “shaking” pattern of sperm motility is suggestive of the presence of ASA [31]. Both IgG and IgA can be found in the cervical mucus. Although PCT shows poor results in the presence of ASA, technical problems may also be responsible; thus, caution is urged when attributing poor PCT to immunologically hostile mucus.

The in vitro sperm-cervical mucus contact (SCMC) test could be also used to evaluate the presence of ASA in cervical mucus. During the SCMC, aliquots of cervical mucus and liquefied semen are mixed and examined for the characteristic "shaking" pattern of sperm motility. The test is considered positive if more than 25 % of spermatozoa display the motility shaking pattern. In a study on 17 couples who repeatedly demonstrated unexpected poor postcoital tests, 15 of them revealed a positive SCMC test [15]. Therefore, the SCMC test can be considered as a reliable screening test for the detection of ASA among infertile couples.

13.3.3 Immunofluorescence and Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay (ELISA) technique has been adapted to quantitatively assess the presence of ASA. ELISA combines the specificity of the antigen-antibody reaction with the continuous degradation of chromogenic substrate by an enzyme to amplify the sensitivity of the reaction. Numerous materials and methods have been used as variations for the ELISA procedure: solid-phase materials (silicon rubber, glass), carriers (tubes, beads, disks), enzymes (alkaline phosphatase, horseradish peroxidase), substrates (p-nitrophenyl phosphate), and different wash solutions [3]. Other variables include sperm concentration, type of sperm fixation, blocking agents, serum, and seminal plasma dilutions. The complexity, instrumentation, and expense of the ELISA have prevented its widespread use in the workup of male immunological infertility.

The use of flow cytometry has been reported to detect sperm-bound antibodies and to quantitate the sperm antibody load (antibody molecules/spermatozoa). Following staining of the washed sperm samples, dead sperm are excluded with fluorescein-isothiocyanate-conjugated F(ab')2 fragments of anti-IgG and IgA antibodies by the use of calibration standards. Flow cytometry has the potential reliability and objectivity to quantitate sperm antibodies; therefore, the sperm antibody load can be used to compare different patients or to follow up the progression of the same patient [25]. Similar to ELISA, flow cytometry is not currently widely used for the detection of ASA due to its complexity, expense, and instrumentation requirement. In the same context, the agglutinin radiolabeled antibodies assay for the detection and quantitation of ASA is of limited use. This method is also limited by an inability to determine specific ASA location, expense, and reliance on highly skilled labor [18].

13.3.4 Mixed Antiglobulin Reaction (MAR) Test

The MAR test has been developed to detect surface ASA [22]. It is based on a modification of the famed Coombs test which was described in1956 by Coombs et al. [8]. The simple initial version of the assay entailed mixing of three ingredients as single drop and covering them with a cover slip. The semen sample is mixed with a suspension of group O, Rh-positive, human red cells of R_1 R_2 type, sensitized with

human IgG in addition to rabbit or goat, undiluted, monospecific anti-IgG antiserum. The reaction is then observed after 10 min of incubation. Since the red cells are coated with IgG as well as the sperm cells if they have antibodies on them, the added anti-IgG antiserum will then link together the two kinds of cells. Agglutination can be seen under a light microscope as mixed clumps of spermatozoa and red blood cells with a slow "shaky" movement.

Results of the MAR test are indicated as percentages of motile spermatozoa incorporated into the mixed agglutinates. The site of attachment could be also noted. No interpretation of the test was given unless agglutination of red blood cells and the presence of sufficient motile spermatozoa are observed. A MAR test was considered positive and of clinical significance when >50 % agglutination is seen [1]. The advantages of the MAR test are that it can be applied directly to untreated semen samples and the results can be obtained within few minutes, which renders the assay quick, simple, and repeatable. The MAR test correlates with most other sperm antibody tests, e.g., SIT and IBT [22]. Although MAR test is considered an ideal method for screening of ASA, it is not without limitations [30]. The assay cannot be used in patients with oligozoospermia, asthenozoospermia, and azoospermia. Also it must be performed on a fresh sample and can be difficult to quantitate due to the presence of debris, semen viscosity, mucus, and microbial factors.

Commercially available sperm MAR kit use an antiserum against human IgG to induce mixed agglutination between antibody-coated latex beads conjugated with human IgG [24]. The sperm MAR kit can be considered a superior alternative to erythrocyte MAR since it is time and cost effective (Fig. 13.1). One formulation of the kit contemplates the assessment of IgA as well as IgG classes. The assay can be

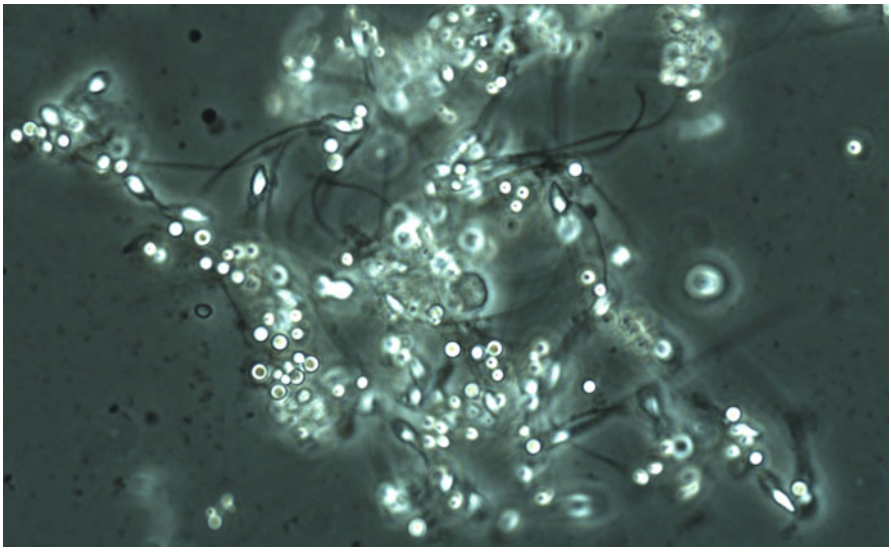

Fig. 13.1 Positive mixed antiglobulin reaction (*MAR*) test. Raw semen sample with latex beads coated with IgG seen bound to sperm surfaces mainly tails (phase contrast, 40×)

successfully used for the evaluation of male partners of infertile couples if included routinely in semen analysis [36]. An indirect MAR, i.e., using serum/seminal plasma samples and donor spermatozoa, can be considered in cases with azoospermia. However, it has been reported as difficult to interpret. Therefore, ASA should be rather detected using another approach in these cases.

13.3.5 Immunobead Test (IBT)

The IBT has been described as a relatively simple, inexpensive procedure, which takes less than 30 min to perform. Similar to the MAR test, it is very convenient, utilizing only a bench centrifuge, light microscope, and latex beads coated with anti-human IgG, IgA, and IgM [14]. IBT allows the determination of the antibody class attached to spermatozoa, the localization on the spermatozoa, and the proportions of spermatozoa coated with antibody [14]. The immunoglobulin class detected can be of clinical importance (Fig. 13.2).

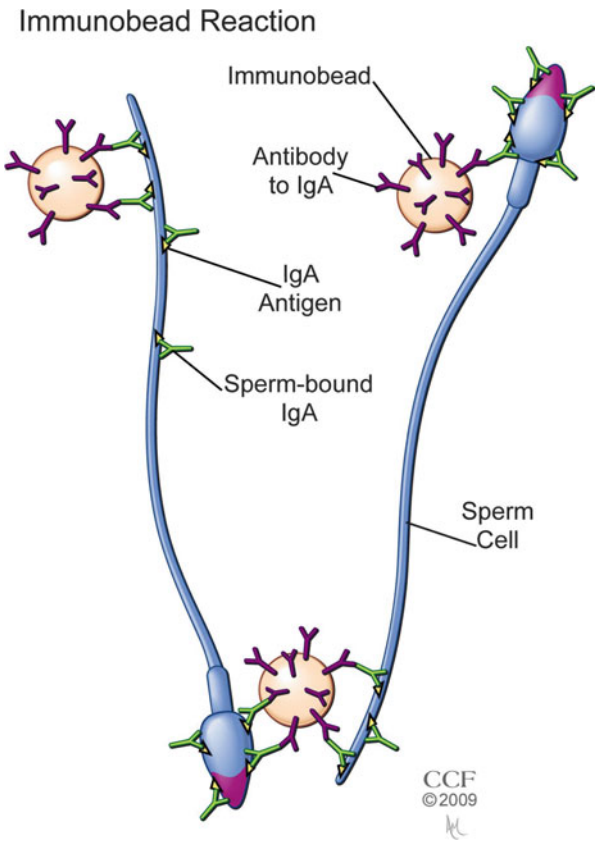

Fig. 13.2 Immunobeads are polyacrylamide spheres with covalently bound rabbit anti-human immunoglobulins. The test is considered positive if ≥20 % of motile spermatozoa have immunobead binding and is considered clinically significant when at least 50 % of the motile spermatozoa are coated with immunobeads

Prior to conducting the assay, spermatozoa must be washed to discard any free immunoglobulins, which may be in the seminal plasma and which, if present, would alter the assay results. Thereafter, sperm concentration is adjusted to 10–25 × 10^6 motile sperm/mL to optimize the microscopic assessment of sperm. These adjustments are occasionally necessary if the sample is oligozoospermic or asthenozoospermic. The IBT can also be conducted indirectly on reproductive fluids such as seminal plasma, follicular fluid, cervical mucus, as well as serum.

The intra-assay reproducibility of the indirect IBT was evaluated by testing aliquots of ASA-positive sera from two patients against the same donor sperm sample. The interassay reproducibility was also evaluated by testing a positive serum sample first with different sperm samples from the same donor and second with sperm samples from different donors. The results of those experiments showed that the indirect IBT has very low intra-assay variation and a high interassay variability [13]. Therefore, both the direct and indirect IBT can be considered as reliable, specific tests for the detection of sperm-bound antibodies and sperm antibodies in reproductive fluids and serum [24]. IBT was routinely applied in many andrology laboratories for the detection of ASA. However, a current lack in commercially available coated latex beads has led to its discontinuation.

13.4 Comparison of ASA Tests

The comparison between the MAR test and the IBT is of particular importance since both assays appear to be the most commonly used [37]. The two testing protocols are designed to detect immunoglobulins on the sperm surface; however, the MAR test is specific for the secretory IgA which is present in semen, while the IBT may cross-react with nonsecretory IgA present in serum [29]. To initiate a comparison, indirect tests for ASA using the commercially available MAR and IBT kits were applied to a panel of sera whose reactions in the TSAT, GAT, and SIT were well characterized. The results from assessments of 30 sera confirmed a significant correlation between the GAT, MAR, and IBT. When sera were titrated, the IBT proved to be slightly more sensitive than the GAT, while the MAR test was slightly more sensitive than the IBT [24].

A different comparative study between the IBT and the MAR test has also shown a high degree of agreement between both assays; however, the former was less accurate than the latter [29]. A similar study reported that the standard MAR protocol (direct test of unwashed semen) was found to be more sensitive than IBT on washed sperm. Other advantages for the MAR include not requiring washing the spermatozoa free of the seminal plasma, which makes it easier and faster than the IBT. It also uses less semen volume and could be applied to samples with a lower sperm concentration compared to the IBT [2, 34]. In contrast, when the MAR test was performed on washed sperm or with an indirect antibody transfer from serum or seminal plasma, the results gave mostly lower values for bead binding in comparison with the IBT. Therefore, the MAR test can be considered mostly

suitable in direct assays employing unwashed ejaculates making it easier to incorporate in routine semen analysis. However, it is prudent to confirm its positive results by IBT [20].

Cross-inhibition studies have revealed high specificity between positive IBT and the presence of membrane-bound immunoglobulin. Good correlations have been reported also between the IBT and other immunoassays such as PCT and sperm-cervical mucus compatibility assays [33]. Poor correlations have been reported between the IBT and sperm agglutination test, which implies that sperm agglutination may occur due to other nonimmunological factors [14]. Therefore, false-positive results may occur with agglutination tests in the absence of ASA. Finally, it is of importance to note that there are several pitfalls associated with comparisons between the different methods of ASA detection. Many methods rely on subjective determinations and variable specimen preparations. In addition, the sensitivity and specificity for each of the testing modalities vary widely [19].

13.5 Interpretation and Significance of ASA Tests

There is sufficient evidence supporting the hypothesis that ASA play a role in selected cases of infertility. The prevalence and magnitude of this role remains not well defined. In the clinical context of male infertility, the MAR test and the IBT are currently recommended for the detection of ASA. The current consensus indicates that a semen sample is to be considered immuno-compromised if more than 50 % of spermatozoa show binding in the MAR test or the IBT [1, 37].

The identification of ASA in a given sample does not necessarily indicate other inherent defects. An attempt to correlate results of MAR test with other defects in the seminal fluid revealed a significant correlation between a positive MAR test and spontaneous sperm auto-agglutination [7]. No correlations were observed, however, between test positivity and sperm concentration, motility, morphology, macroscopic features, or leukocytes concentration. Contradictory findings were identified in a different trial that correlated the results of the IgG MAR test with the semen analysis parameters of 1176 infertile males [27]. The test was only positive in 3.1 % of the cases. The positive IgG MAR test proved to correlate significantly both with the number and motility of spermatozoa. Whether the detection of ASA is associated with other deficiencies in the semen analysis or not should not infringe on the importance of the assay, which appear to be of significant value in identifying the etiology of infertility in some cases.

In a study that evaluated ASA levels in men presenting with a history of infertility, MAR test results were found to be positive in 10 % of 484 men with normal sperm counts, 23 % of 78 with low sperm motility, and 15 % of 128 with low counts. Therefore, the MAR test may be considered as a part of the routine semen analysis, since the presence of IgG antisperm antibodies can be established in about 10 % of men who might otherwise be passed as normal. In support, the evaluation of patients whose infertility remains unexplained (by routine physical and laboratory

investigations) exhibits significantly elevated ASA levels in 18 % of males compared to fertile individuals. Thus, the identification of autoimmune imbalance may help to resolve some cases of unexplained infertility [12].

Conclusions

The assessment of ASA in the context of infertility has not been devoid of controversies. It is duly noted that "neither a specific antigen (s) nor a superior antibody detection assay exists, although both are requisite to an understanding of the significance of ASA production and for the purpose of infertility reduction" [10]. The only agreed upon current indication for performing an ASA detection assay appears to be unexplained infertility. Published data support the use of either IBT or MAR as the only reliable tests capable of assessing the presence of ASA in a clinical setting. The MAR test is easier to perform and may be more sensitive but is not suitable for testing of serum or plasma using an indirect approach.

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