Detection of Antispermal Antibodies in Female Sera

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The levels of antispermal antibodies in the sera of women varied within a wide range when spermatozoa from different donors were used. This was probably due to expression of different antigenic structures on the surface of spermatozoa of fertile and sterile men and their different specificity for the tested female serum. Spermatozoa from different donors did not influence the detection of antispermal antibodies in the sera of sterile men. The use of spermatozoa from the husband and a fertile donor is recommended for identification of antispermal antibodies in female serum when examining an infertile couple.

Key Words: *antispermal antibodies; infertility*

Detection of antispermal antibodies (ASAB) is important for diagnostic of immunological infertility. The indirect mixed antiglobulin reaction (MAR) is widely used for detecting ASAB in the serum [1]. It should be remembered, that MAR allows one to assess the proportion of spermatozoa carrying antigens specific for the tested serum ASAB on their surface. Obviously, the use of spermatozoa containing no antigens specific for a certain serum can lead to false-negative results during detection of ASAB in the female sera by MAR with spermatozoa of different donors.

MATERIALS AND METHODS

Ejaculate was incubated for 30 min at room temperature for thinning and analyzed by the direct MAR test [1]. In further studies, only spermal samples without ASAB were used. The fraction of actively moving spermatozoa was obtained by the swim up method [2]. For indirect MAR test, the sera were warmed for 30 min at 56°C in a water bath in order to inactivate the complement. A suspension of actively moving spermatozoa (25 μl) was added to the tested serum (25 μl) and incubated at 37°C for 1 h. Then the cell suspension was layered onto Percoll and centrifuged for 5 min, after which 10 μl of cell suspension was collected from the sediment and mixed on a slide with 10 μl of latex corpuscles coated with human IgG and 10 μl rabbit antiserum (Orto Diagnostic). The results were examined under a phase-contrast microscope at magnification 400. Mobile latex-agglutinated spermatozoa per 100 mobile spermatozoa in the test (MAR%) were calculated. The results were recorded 2-3 min after the test had been prepared and then repeatedly in 10 min.

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RESULTS

The results of indirect MAR test of sterile female sera (n=21) with husband's and donor's spermatozoa as the antigens are compared. Figure 1 shows the ASAB IgG levels. Four types of sera were identified: 1) with ASAB detected only in the presence of donor spermatozoa (n=4); 2) with ASAB detected only by husband's spermatozoa (n=3); 3) with ASAB detected by both donor's and husband's spermatozoa, but the MAR% to husband's spermatozoa higher than the MAR% to donor spermatozoa (n=11); and 4) without ASAB to husband's or donor's spermatozoon antigens (n=3).

Thus, the MAR% in 11 out of 21 tests (52%) with husband's spermatozoa was higher than the

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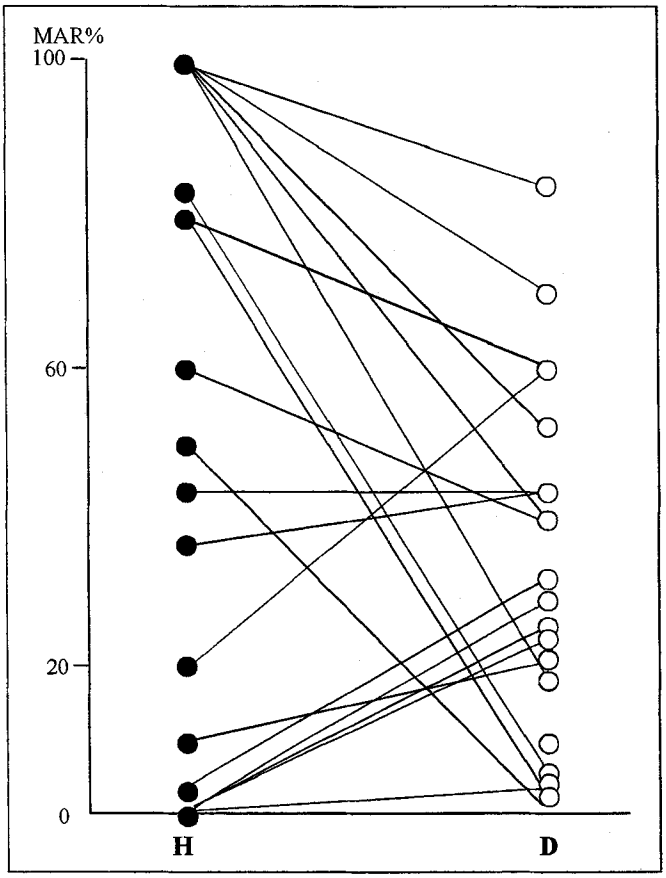

Fig. 1. Detection of antispermal antibodies in the sera of infertile women using husband's (H) and donor's (D) spermatozoa.

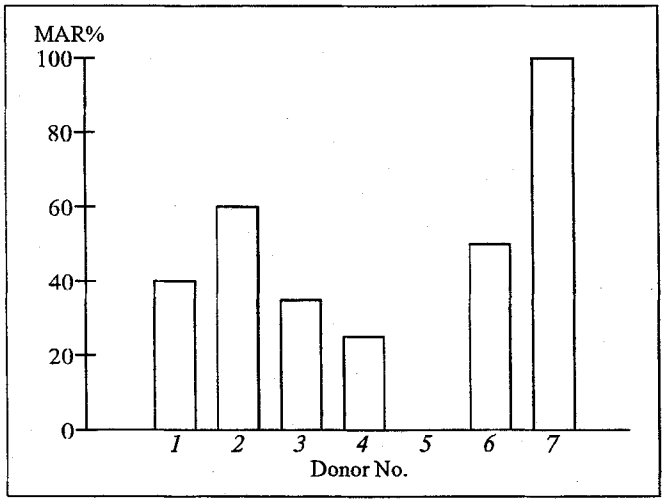

Fig. 2. Detection of antispermal antibodies in the serum of infertile women using spermatozoa from different donors.

MAR% in tests with donor's spermatozoa, and in 3 (14%) cases the use of donor's spermatozoa yielded false-negative results. On the other hand, false-negative results were obtained in 4 (19%) tests with husband's spermatozoa.

ASAB to spermatozoa from 7 donors were tested in the sera of 10 sterile women and 5 sterile men (Fig. 2). In 9 women, the results of MAR varied within a wide range, the coefficient of variations being 50-75%. Figure 2 shows the results of ASAB detection in a serum in which the MAR% varied from 0 to 100% (cv=69%). Only in one woman the MAR% was 100% with spermatozoa of all 7 donors. In contrast to female sera, the MAR% of sera of men with immunological sterility was 100% in all the cases (cv=0%).

Thus, the ASAB values in female sera were different not only with husband's and donor's spermatozoa, but also with spermatozoa of different donors. Therefore, the differences in the results of MAR test are not due to the specific reaction to husband's spermatozoon antigens.

Numerous antigenic structures are localized on the surface of spermatozoa [4]. Some identified antigens (28, 38, 48, 60, and 68 kD) are present on spermatozoa of all men, no matter whether fertile or not [5]. Antigens with molecular weights 76, 25, and 46 kD are present only on the spermatozoa of fertile men [6], whereas 41, 43, 30, 35, 52, and 71 kD antigens are detected only on the surface of spermatozoa of sterile men [3].

Presumably, men produce ASAB to antigens expressed on the surface of all spermatozoa, therefore assessment of ASAB using donor spermatozoa as antigens shows the same number of ASAB-positive cells in male sera. Women probably produce ASAB to specific antigens present on spermatozoa of some men. That is why the results of MAR and other methods for detecting ASAB in female sera may depend on the expression of these specific antigens on donor spermatozoa.

Antigens which are not expressed in fertile men are probably present on the surface of spermatozoa of infertile men, and detection of ASAB only to husband's spermatozoa does not provide sufficient information on the state of antispermal immunity of a woman; therefore, we recommend using both husband's and fertile donor's spermatozoa for detecting ASAB in female sera in examinations of infertile couples.

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