EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Adaptation of Flow Cytofluorimetry for Detection of Antispermal Antibodies in the Serum and Peritoneal Fluid of Women

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Antispermal antibodies were detected by flow cytofluorimetry and mixed antiglobulin reaction (MAR). The results of the two methods significantly correlated regarding serum IgG antispermal antibodies under conditions ruling out capacitation of spermatozoa. There were no significant differences in the content of antispermal antibodies in the sera of fertile, pregnant women, women with endometriosis and adhesions, or in the peritoneal fluid of fertile and sterile women. In women with endometriosis and adhesions the level of antispermal antibodies in the peritoneal fluid was significantly higher than in the serum. The use of flow cytofluorimetry markedly improved the detection of antispermal antibodies in sterile women.

Key Words: *flow cytofluorimetry; antispermal antibodies; serum*

Despite numerous studies of the role of immune factors in sterility, the data on the contribution of antispermal antibodies (ASAB) to reproductive disorders in women are contradictory. These contradictions are due to the absence of a universal objective method for ASAB detection. Mixed antiglobulin reaction (MAR) is recommended by the WHO for identification of ASAB. However, this test is subjective and requires a labor-consuming microscopic analysis.

Recently, flow cytofluorimetry (FCFM) was adapted for detecting ASAB in the ejaculate, male sera, and cervical mucus; this method possesses advantages over other methods of ASAB detection [1,4-6]. The aim of this study was to explore the possibility of using FCFM for detecting ASAB in female serum and peritoneal fluid.

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MATERIALS AND METHODS

Ejaculate was collected from healthy donors, incubated for 30 min at ambient temperature, and the mobile spermatozoa fraction was obtained by the swim-up method [3]. For indirect MAR test, serum and peritoneal fluid samples were warmed at 56°C in a water bath for 30 min in order to inactivate the complement. The suspension of active mobile spermatozoa (25 μl) was added to 25 μl of test sera and peritoneal tissue samples and incubated at 37°C for 1 h. Then the mixture was layered onto Percoll and centrifuged for 5 min, after which 10 μl cell suspension was collected and mixed on a glass slide with 10 μl latex corpuscles coated with human IgG and 10 μl rabbit antiserum (Orto Diagnostic).

The samples were examined under a phase-contrast microscope at magnification 400. The number of mobile spermatozoa agglutinating latex particles

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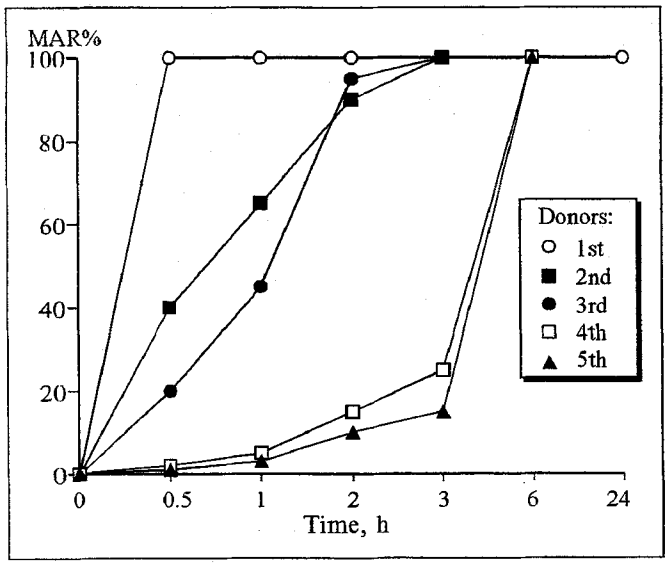

Fig. 1. Relationship between binding of serum antispermal antibodies of infertile women to spermatozoa and the duration of incubation.

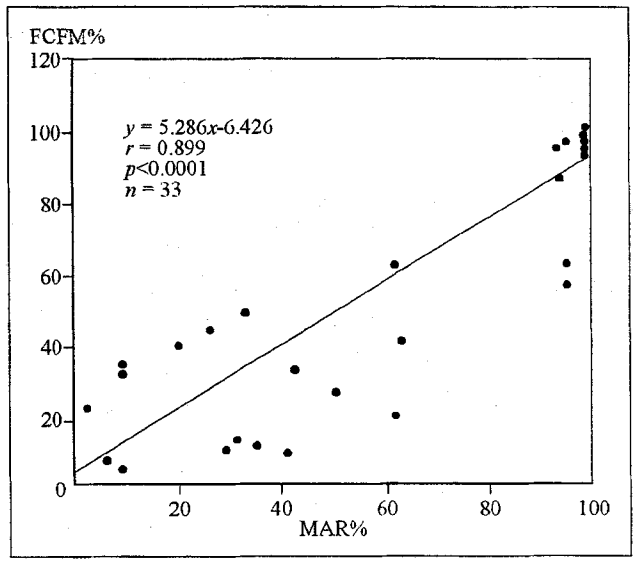

Fig. 2. Correlation between the results of detection of antispermal antibodies in the sera of infertile women by flow cytofluorimetry (FCFM) and MAR test.

per 100 mobile spermatozoa in a sample (MAR%) was calculated.

The results were recorded 2-3 min after the sample had been prepared and then again after 10 min. For detecting IgG on the surface of live spermatozoa by FCFM, 4 μl suspension of spermatozoa obtained by the swim-up method (10^7 cells/ml) were added to the sample (28 μl inactivated whole serum or peritoneal fluid) and incubated at 4°C on ice for 1 h. After incubation, the cells were washed twice in Dulbecco's phosphate buffer, pH 7.4. Resuspended sediment (25 μl) was placed into a tube with 1 μl monoclonal murine antibodies to human IgG labeled with fluorescein isothiocyanate (FITC, Sigma). After a 15-min incubation at room temperature, the spermatozoa were twice washed in Dulbecco's phosphate buffer, and 100 μl of buffer was added to the precipitate.

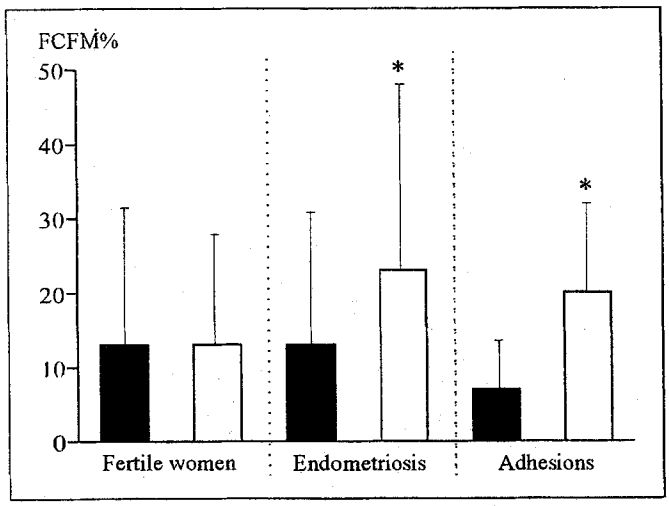

Fig. 3. Content of antispermal antibodies in the sera (dark bars) and peritoneal fluid (light bars) of women, detected by flow cytofluorimetry (FCFM). *p<0.05 *vs.* the serum.

Cell viability was assessed by propidium iodide staining [2]. Final concentration of propidium iodide in cell suspension was 2 μg/ml. Analysis was carried out on a Becton Dickinson FACScan flow cytofluorimeter. Each sample contained 5000 cells; the percentage of ASAB-positive spermatozoa (FCFM%) was assessed. ASAB-negative spermatozoa incubated in medium 199 with 3% bovine serum albumin in the presence of FITC-labeled antibodies to human IgG served as the control.

RESULTS

The expression of antigens on the surface of spermatozoa is liable to change: while the cells are incubated in a medium containing serum components, capacitation takes place: some antigens appear, others disappear. For assessing the relationship between the duration of incubation of spermatozoa with serum and the results of ASAB detection, serum specimen from an infertile woman was incubated with ASAB-negative spermatozoa of 5 fertile donors for 30 min—24 h (Fig. 1).

Figure 1 shows that after 30 min of incubation, MAR% was 100% with the spermatozoa of donor 1. In other cases the capacitation process also led to an increase in MAR%, and after 6-h incubation MAR% was 100% in spermatozoa of all 5 donors. Thus, incubation of spermatozoa with serum required for routine detection of ASAB can detect antigens which appear only during incubation.

Obviously, ASAB to native spermatozoa should be detected under conditions ruling out the process of capacitation.

Along with the MAR-test, an objective method (FCFM) was used for detecting ASAB. The results of detection of serum IgG ASAB by the indirect MAR test and standard FCFM were in significant correlation (n=33, r=0.899, p<0.0001) (Fig. 2). The minimum FCFM% corresponding to MAR=100% was 79% for the 95% level of significance, for MAR=40% (recommended by the WHO as the minimum clinically significant level of ASAB), it was 29%.

These data demonstrate that FCFM can be used for detecting ASAB in the female sera. In contrast to MAR test, FCFM can be performed with immobile spermatozoa, which is very important in the presence of ASAB inhibiting the spermatozoon motility.

FCFM quantifies both the ASAB-positive spermatozoa (FCFM%) and ASAB on their surface. Moreover, FCFM involves no subjective microscopic examination and routine cell counting, which is particularly important for simultaneous testing of large sample series. As capacitation increases, the number of ASAB-positive spermatozoa, FCFM for the detection of ASAB in female sera and peritoneal fluid samples was carried out under conditions precluding capacitation: incubation was carried out at 4°C.

Figure 3 shows ASAB detection by FCFM under conditions precluding capacitation with spermatozoa of the same donor. There are no significant differences in the levels of ASAB in the sera of fertile women (n=10), pregnant women (n=10), women with endometriosis (n=20), and adhesions (n=15), and in the peritoneal fluid of fertile and sterile women. The content of ASAB in the peritoneal fluid of women with endometriosis and adhesions was 1.8 (p<0.05) and 2.8 times (p<0.01) higher, respectively, that in the serum. On the whole, in 35 sterile women, ASAB in serum and/or peritoneal fluid were detected by the standard MAR test in 54% of cases and in 29% of cases by FCFM without capacitation. Obviously, FCFM test without capacitation is a more effective tool for the detection of ASAB in sterile women, yielding a lesser number of false-positive results.

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