5. V. I. Votyakov and A. G. Kolomiets, Vestn. Akad. Med. Nauk SSSR, No. 5, 3 (1988).
6. D. D. Zerbina and L. L. Lukasevich, Arkh. Patol., No. 8, 72 (1984).
7. A. G. Kolomiets, S. A. Guzov, P. G. Rytik, and N. D. Kolomiets, Vopr. Virusol., No. 3, 369 (1987).
8. A. G. Kolomiets, S. A. Guzov, I. I. Protas, et al., Klin. Med., No. 8, 127 (1987).
9. S. S. Marennikova, G. R. Matsevich, N. T. Chekunova, et al., Vopr. Virusol., No. 1, 59 (1986).
10. M. K. Nedz'ved', I. I. Protas, V. I. Votyakov, et al., Arkh. Patol., No. 10, 60 (1982).
11. A. K. Shubladze and T. M. Maevskaya, Herpes [in Russian], Moscow (1971).
12. L. Aloe, Int. J. Develop. Neurosci., **5**, No. 4, 357 (1987).
13. R. A. Chase, J. C. Pottage, M. H. Haber, et al., Rev. Infect. Dis., **9**, No. 2, 329 (1987).
14. A. Gerritzen and K. E. Schneweis, Zbl. Bakt. Mikrobiol. Hyg., **267**, No. 1, 125 (1987).
15. R. J. Klein and S. M. Czelusniak, Proc. Soc. Exp. Biol. (New York), **184**, No. 1, 50 (1987).
16. A. Matoba, Pediat. Infect. Dis. J., **3**, No. 4, 358 (1984).
17. I. Moldy, J. Lerman, and R. White, Am. J. Dis. Child., **135**, No. 1, 45 (1981).
18. N. A. Sanjuan and E. F. Lascano, Arch. Virol., **91**, No. 3-4, 329 (1986).
19. W. Worthmann and R. Thouet, Munch. Med. Wschr., No. 11, 262 (1985).

DETECTION OF ANTISPERM ANTIBODIES ON THE SURFACE OF LIVING SPERMATOZOA BY FLOW CYTOMETRY

M. A. Nikolaeva, G. V. Ter-Avanesov, L. N. Terekhina, T. Ya. Pshenichnikova, and G. T. Sukhikh

UDC 612.616.2.017.1:083.33+ 616.697-07:616.153.962.4-097-02:616.69-008.8

Key words: flow cytometry; antisperm antibodies

The detection of antisperm antibodies (ASAB) is an important stage in the diagnosis of sterility. Most methods used to detect antisperm antibodies (sperm agglutination [1], immobilization [2], enzyme immunoassay [3], and radioimmunoassay [4]) are aimed at detecting antibodies present in seminal fluid, cervical mucus, or serum, but they do not enable the quantity of antibodies on the surface of spermatozoa to be estimated; the mixed agglutination reaction test [5] and the IBT test [6] reveal the localization of antibodies of different isotypes actually on the surface of spermatozoa, but cannot be used to determine the quantity of antibodies bound with the cells. The aim of this investigation was to estimate the quantity of antibodies of different isotypes (IgG, IgA, and IgM) on the surface of living spermatozoa by the flow cytometry method (FCM).

EXPERIMENTAL METHOD

To determine ASAB in seminal fluid spermatozoa were washed with phosphate buffer (PB, pH 7.4) and incubated with mouse monoclonal antibodies to human IgG, IgA, and IgM and then treated with FITC-labeled antibodies to mouse Ig (Beckton, Dickinson, West Germany). Propidium iodide (PI), which stains only dead cells [6], was added to the suspension of cells in PB. The "Facscan" cytofluorometer (Becton Dickinson) was used for analysis, 10,000 living cells being studied in each sample (dead cells were eliminated from the analysis by the computer on the basis of their staining with PI). For quantitative assay of the different classes of Ig on the surface of the spermatozoa, the mean value of x, expressing fluorescence in conventional units, was determined for each histogram of distribution of the cells according to their green fluorescence, and compared with the value of

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 379-382, April, 1990. Original article submitted October 20, 1989.

 0007-4888/90/0004-0496$12.50

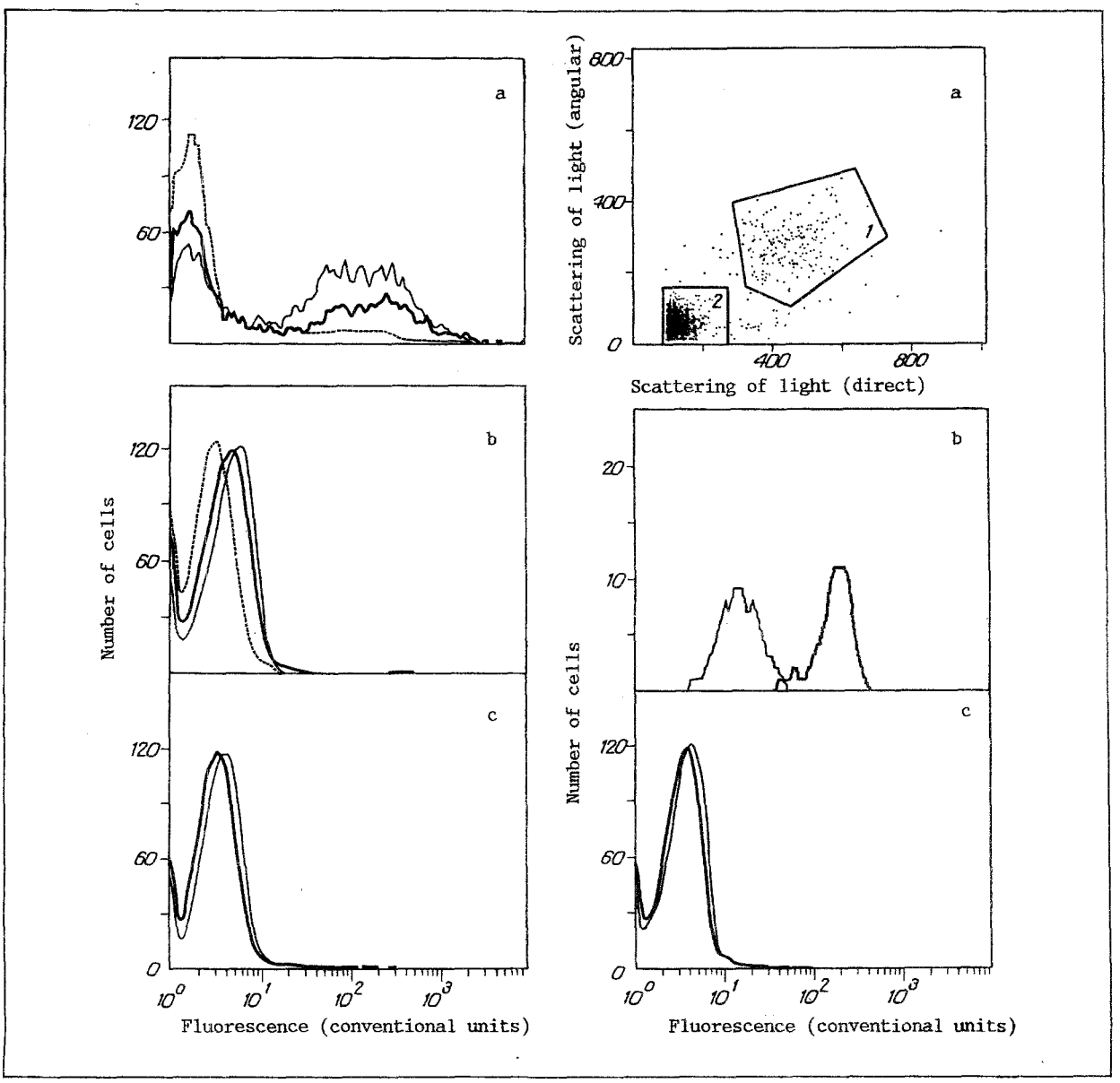

Fig. 1

Fig. 2

Fig. 1. Histograms of distribution of spermatozoa by intensity of green fluorescence after incubation with monoclonal antibodies to human IgG and IgA and with FITC-labeled antibodies to mouse Ig. To determine nonspecific binding the spermatozoa were stained only with FITC-labeled antibodies to mouse Ig; a, b) specimens of sperm with negative MAR test.

Fig. 2. Analysis of ASAB (IgA) content on surface of spermatozoa after their incubation with cervical mucus. a) Distribution of cells in coordinates of direct and angular scattering of light. "Window" 1) cells of cervical mucus; "window" 2) spermatozoa; b) histogram of distribution of cells of cervical mucus by IgA content on their surface; c) histogram of distribution of spermatozoa by IgA content on their surface.

x of the histogram obtained when the cells were treated only with the second antibodies (the control of nonspecific binding). The ratio served as an index of the content of antibodies on the surface of the cells and henceforward it will be designated A. In the absence of antisperm antibodies $A \leq 1$, in the presence of ASAB, $A > 1$. If ASAB were not present on the surface of the spermatozoa, they were used to detect antibodies in cervical mucus and serum. For this purpose 100 μl of seminal fluid was washed with PB. The washed cells were treated with 100 μl of serum or cervical mucus and incubated at 37°C for 1 h. After incubation the spermatozoa were washed with PB and used to determine ASAB, as described above.

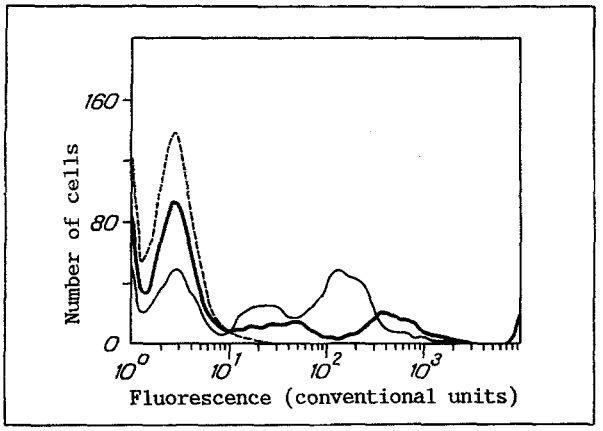

Fig. 3. Analysis of ASAB content on surface of spermatozoa after their incu bation with cervical mucus.

EXPERIMENTAL RESULTS

When 20 seminal fluids were tested, IgG were discovered in two cases by both MAR and FCM tests. The MAR test showed that 30% of spermatozoa carry IgG on their surface. FCM revealed the bimodal character of the histogram of green fluorescence, corresponding to two cell subpopulations: 1) spermatozoa with IgG on their surface (27%) and 2) spermatozoa not carrying antibodies (73%; Fig. 1a). In the second case the MAR test revealed 57% of spermatozoa with antibodies, but the character of the distribution of the cells by green fluorescence in this case was unimodal (Fig. 1b). In 18 cases ASAB could not be found, and the value of A for IgG, IgA, and IgM did not exceed 1 in all 18 (Fig. 1c). The intensity of green fluorescence (FCM test) and the number of spheres on the surface of the spermatozoa (MAR test) reflect the content of IgG on the surface of the spermatozoa. In the first case, with each spermatozoon more than 10 spheres were bound, whereas the value of A determined in the sperm immobilization test for the IgG-carrying population was 120. In the second case 50% of spermatozoa contained one or two spheres on their surface, and the value of the ratio A for the whole population of spermatozoa was 1.2. It can be tentatively suggested that when there are few antibodies on the surface of the spermatozoa the FCM test may turn out to be more sensitive than the MAR test. It was found by the FCM test that specimens with antisperm IgG also contained antisperm IgA (Figs. 1 and 2). In the first case the number of IgA- and IgG-positive cells differed significantly (27% and 47% respectively). The content of IgG and IgA bound with the surface of the spermatozoa also differed: in the first case $A = 178$ and $A = 69$; in the second case $A = 1.4$ and $A = 1.5$.

When ASAB in the cervical mucus were determined, it had to be borne in mind that cells present in cervical mucus may also carry IgG, IgA, and IgM on their surface. By computer analysis it is possible to analyze separately subpopulations of spermatozoa and cells of the cervical mucus by setting the "windows" on the two-dimensional distribution in coordinates of direct and angular scattering of light (Fig. 2a). Results of analysis of cells after incubation of the spermatozoa with cervical mucus are given in Fig. 2. They show that the cell population with a high IgA content on the surface ($A = 200$) is represented by two comparatively large cells of the cervical mucus (Fig. 2b), whereas spermatozoa do not contain ASAB on their surface (Fig. 2c). During the subsequent analysis of ASAB in the cervical mucus, only the spermatozoal population was analyzed by setting the "window."

During analysis of 13 samples of cervical mucus ASAB were found in three cases. The results of the FCM test are shown in Fig. 3, and reveal heterogeneity of the subpopulations of spermatozoa after incubation with cervical mucus. On analysis of 13 sera, ASAB only of the IgM class were found in seven, and those of the IgG, IgA, and IgM classes in one serum; heterogeneity of the spermatozoa was not found in any single case. Subsequent analysis of the sera by the sperm immobilization method completely confirmed the results of the FCM test: in all sera with ASAB the sperm immobilization index exceeded 2, evidence of the presence of sperm immobilizins.

Methods of detection of ASAB widely used at the present time have important limitations. The sperm immobilization method [2] cannot be used to detect ASAB belonging to the IgA class [7], and it also leads to false-positive reactions when agglutinins are present. The use of enzyme immunoassay and radioisotope methods does not enable the heterogeneity of the population of spermatozoa for the ASAB content on their surface to be assessed. The MAR and IBT tests cannot be used for

quantitative assay of Ig on spermatozoa. By the use of the FCM test it is possible to compare numbers of ASAB of different isotypes on the surface of the spermatozoa during their incubation in various biological fluids (seminal plasma, serum, cervical mucus, peritoneal and follicular fluids, intrauterine contents). The discovery of antibodies, moreover, is also possible in the absence of motility, in viable spermatozoa. The method is quantitative, objective, readily reproducible, and can be used in clinical practice for the diagnosis and treatment of sterility.

LITERATURE CITED

1. S. Kibrick, D. L. Belding, and B. Merill, Fertil. Steril., **3**, 430 (1952).
2. S. Isijima, K. Tsuchiya, K. Koyama, and C. Tanaka, Am. J. Obstet. Gynecol., **112**, 199 (1972).
3. S. Paul, V. Bauklov, and L. Mettler, J. Immunol. Meth., **56**, 193 (1983).
4. G. G. Haas, O. B. Gines, and A. D. Schreiber, New Engl. J. Med., **303**, 722 (1980).
5. A. Hinting, I. Vermeulen, and F. Comhaire, Fertil. Steril., **49**, 1039 (1988).
6. H. Meinertz and R. Bronson, Am. J. Reprod. Immunol. Microbiol., **18**, 116 (1988).
7. R. Bronson, G. Cooper, and D. Rosenfeld, Fertil. Steril., **42**, 171 (1984).