DETECTION OF ANTISPERM ANTIBODIES BY IMMOBILIZATION AND CYTOTOXICITY TESTS USING FLOW CYTOMETRY

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Detection of antisperm antibodies (ASAB) is an important stage in the diagnosis of infertility. The most widely used methods of determination of ASAB are those of sperm immobilization [1] or sperm cytotoxicity [2], based on the immobilizing and (or) cytotoxic action of ASAB on spermatozoa, and involving counting the number of immobilized or dead spermatozoa after their incubation in test serum in the presence of complement. However, an important disadvantage of these methods is their subjectivity, the need for skill in order to reach accuracy of estimation of the number of motile and nonmotile spermatozoa in a sample, the laboriousness of the microscopic investigation and, consequently, the impossibility of testing a large number of specimens.

The aim of this investigation was to overcome these disadvantages by using the rapid and objective method of flow cytometry (FCM) to count dead or nonmotile spermatozoa.

EXPERIMENTAL METHOD

Antisperm antiserum was obtained by immunizing rabbits with seminal plasma and a homogenate of healthy human spermatozoa. In all experiments to detect ASAB, the fraction of actively motile spermatozoa was used. Medium 199 (8 ml) was layered above the surface of the sperm and the specimen was incubated in a CO_2 incubator for 1 h. After incubation, a sample of the cell suspension in medium 199 was taken with a micropipet and used in all subsequent experiments. The action of ASAB was assessed by the standard method of detection of sperm-immobilizing antibodies [1]. For this purpose 25 μl of the test serum (in dilutions from 1:1 to 1:1024) was treated with 25 μl of the suspension of spermatozoa in medium 199 and 5 μl of complement. After incubation at 37°C for 1 h the number of motile spermatozoa was counted under the microscope and the number of living and motile spermatozoa was determined by flow cytometry. To determine viability, the cell suspension was treated with propidium iodide (PI), which penetrates only into dead cells and accumulates there [3]. Motile cells were counted with the aid of Rhodamine-123, which binds with active mitochondria, thereby enabling motile cells to be recorded and their relative number in the sample determined [3]. For this purpose, Rhodamine-123 (10 μg/ml) was added to the cell suspension and incubated at room temperature in darkness for 10 min; the spermatozoa were then washed off with medium 199 and the residue was resuspended in the same medium and incubated in darkness for 45 min. PI (20 μg/ml) was added to the suspension, which was analyzed 5 min later on a "Facscan" flow cytometer (Beckton Dickinson, USA) relative to 10,000 spermatozoa in each sample, at a speed of 500 cells/sec. The cell distribution was analyzed simultaneously

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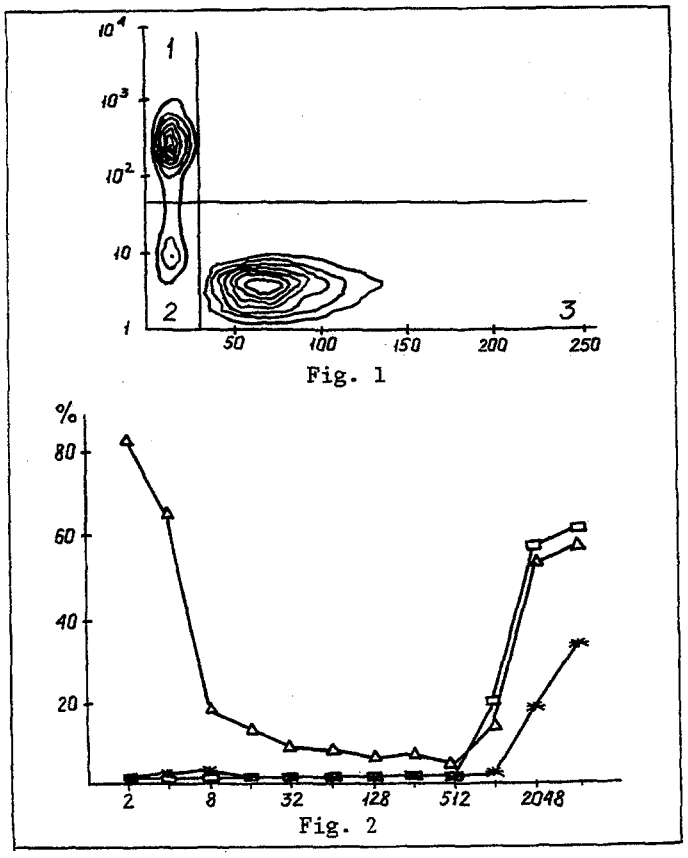

Fig. 1

Fig. 2

Fig. 1. Estimation of number of dead (1), living nonmotile (2), and motile (3) spermatozoa by flow cytometry. Abscissa, intensity of green fluorescence; ordinate, intensity of red fluorescence.

Fig. 2. Viability and motility of spermatozoa after incubation with different dilutions of antisperm serum. 1) Viability of spermatozoa; 2) motility of spermatozoa, determined under the microscope; 3) motility of spermatozoa, determined by flow cytophotometry. Abscissa, dilution of serum; ordinate, number of cells (in percent).

by measuring the intensity of red (PI) and green (Rhodamine-123) fluorescence, which enabled three populations of spermatozoa to be identified: 1) living, motile; 2) living, nonmotile; 3) dead (Fig. 1).

EXPERIMENTAL RESULTS

The results of estimation of motility and viability of the spermatozoa by microscopy and FCM are given in Fig. 2. They show that with an increase in dilution of ASAB from 1:2 to 1:512, no motile spermatozoa could be found either by microscopy or by FCM. Starting with a dilution of 1:1024 motile spermatozoa were found by the two methods, but their number determined by microscopy was less than that revealed by FCM. The reason may be a considerable difference in the total number of spermatozoa counted (100 cells under the microscope, 10,000 cells by FCM), and also the subjective nature of microscopic assessment of motility. On the whole, correlation between the

number of motile cells determined by the two methods was strong ($r = 0.96$; $p < 0.0001$). Estimation of the number of living spermatozoa by FCM showed that with dilutions of 1:2 and 1:4 many spermatozoa (83% and 65% respectively) remained alive, although they were nonmotile (Fig. 2); with an increase in dilution of the serum (from 1:8 to 1:512) the cytotoxic effect was intensified: virtually all the spermatozoa were dead, and starting with a dilution of 1:1024, both the cytotoxicity and the sperm-immobilizing action of ASAB were reduced: the number of living and the number of motile spermatozoa increased with an increase in dilution. Antisperm antibodies, in low dilutions of sera, evidently had an immobilizing, but not a toxic action on the spermatozoa, and this led to false negative results when the cytotoxicity test was used. The study of the action of ASAB-positive sera from sterile women on spermatozoa also revealed inhibition of the cytotoxic action of ASAB on spermatozoa in low dilutions of serum. The possibility that false negative results may be obtained may be the explanation of the absence of correlation between the results of detection of ASAB by the sperm immobilization and cytotoxicity tests in the interlaboratory study carried out by the World Health Organization [4]. This study showed that the sperm-immobilizing action of ASAB in the sera from a number of women was not accompanied by cytotoxicity, in good agreement with our own findings.

Our results are evidence that FCM can be used to detect ASAB by the sperm-immobilization method. Meanwhile, the false negative results of the cytotoxic test, due to inhibition of the spermatotoxic action of ASAB in whole serum or in low dilutions of serum indicate that this method cannot be used to detect ASAB.

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