MICROBIOLOGY AND IMMUNOLOGY

Preparation and Application of Monoclonal Antibodies to Fertility α_2-Microglobulin

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An FAMG-Fertitest-M test-system for quantitation of fertility α_2-microglobulin (FAMG) in biological fluids has been developed. It is based on original monoclonal antibodies against two different epitopes of FAMG. The range of FAMG concentrations reflecting the *in vitro* fertilizing capacity of sperm is determined, which allows one to assess the effectiveness of extracorporeal fertilization in the treatment of infertility.

Key Words: *fertility α_2-microglobulin; monoclonal antibodies; sperm; extracorporeal fertilization*

Fertility α_2-microglobulin (FAMG), a specific protein of male and female reproductive system, is a dimer glycoprotein with molecular weight 52-56 kD. The protein was originally identified as a placental antigen [3]. It was purified and characterized by several independent research groups and designated as PP14, PEP, and EP15. FAMG is present in menstrual blood, amniotic fluid, endometrium, and decidual tissues of the placenta, seminal vesicles, and sperm. Irrespective of a 20-year investigation, the function of FAMG remains unclear. It was reported that this protein acts is a potent immunosuppressor [4]. On the basis of homology with β-lactoglobulin, FAMG can be classified as a secretory protein transporting low-molecular-weight hydrophobic ligands, for example, retinol [5].

Here we describe preparation of anti-FAMG monoclonal antibodies (MAb), development of a test-system for FAMG quantitation in biological fluids, and clinical application of these MAb.

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MATERIALS AND METHODS

Preparation of monoclonal antibodies. Purified FAMG antigen was a generous gift from Dr. D. D. Petrunin (Institute of Physicochemical Medicine, Moscow). Antibody-producing hybridomas were obtained by the standard methods [2]. Female BALB/c mice were immunized with FAMG (10 μg intraperitoneally, 3 times at 4-week intervals). Six weeks after the last injection the mice were given an intravenous infusion of FAMG (10 μg in phosphate-buffered saline). Splenocytes and SP2/0 myeloma cells (10:1) were fused with the use of polyethylene glycol-4000 (Merck). FAMG-specific hybridomas were screened two times by the method of limiting dilutions.

The hybridomas were grown as ascites in pristane-primed BALB/c mice. Monoclonal antibodies were precipitated from the ascites with 50% buffered ammonium sulfate and purified on DEAE cellulose. The MAb isotype was determined by solid-phase immunoenzyme assay with the use of rabbit monospecific antibodies to murine immunoglobulin classes and subclasses (Miles). The specificity of MAb was assessed by indirect immunoenzyme assay using placental α_1-microglobulin, chorionic gonadotropin, α-feto-

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protein, trophoblastic β_1-globulin, human serum albumin, bovine serum albumin, and C-reactive protein.

Solid-phase immunoenzyme assay for hybridoma screening. In order to eliminate MAb against antigen with another conformation, the following variant of immunoenzyme assay was employed. Polystyrene plates were coated with rabbit antibodies to murine IgG (2 μg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.5) at 4°C overnight. Nonspecific sorption was prevented with 1% bovine serum albumin in phosphate-buffered saline (PBS, pH 7.4). Hybridoma-conditioned medium was incubated in these plates for 1 h at room temperature with constant shaking, after which FAMG conjugated with horseradish peroxidase (1:1000 in PBS) was added and incubated for 1 h at room temperature with constant shaking. After each incubation, unbound components were washed with tap water (not less than 5 times). The reaction was developed with 0.05% o-phenylenediamine and 0.01% H_2O_2 in 0.05 M phosphate-citrate buffer (pH 4.7) for 20 min in the dark at room temperature. The reaction was stopped with 1 N H_2SO_4 and read in a Multiskan spectrophotometer at 492 nm.

Double-center immunoenzyme assay for FAMG quantitation. In order to select the MAb pair reacting with different FAMG epitopes, the antibodies were conjugated with horseradish peroxidase by the method [6]. Each MAb was used in different combinations both as primary and detecting antibody according to the protocol described in detail elsewhere [1].

Clinical material. The FAMG concentration was determined in the sperm of 187 male patients at the EKO Moscow Center in the course of 245 attempts to treat infertility by extracorporeal fertilization. Sperm aliquots were frozen at -18°C, thawed for 1 h at room temperature before FAMG determination, which was performed in triplicate. The results were processed statistically.

RESULTS

Seven stable hybridomas producing anti-FAMG MAb have been obtained. All MAb were IgG_1 and did not cross-react with placental and serum proteins.

Double-center immunoenzyme assay showed that these MAb recognize at least two epitopes on FAMG molecule, and 2 out of 7 antibodies recognize the same epitope.

In order to select the optimal MAb pair, we performed parallel determinations of the FAMG concentration in serum and sperm using the standard test-system based on rabbit anti-FAMG monospecific antibodies and different combinations of our MAb. The best coincidence was obtained with 4f8 and 3g12 MAb, the variation coefficient being 4-9%.

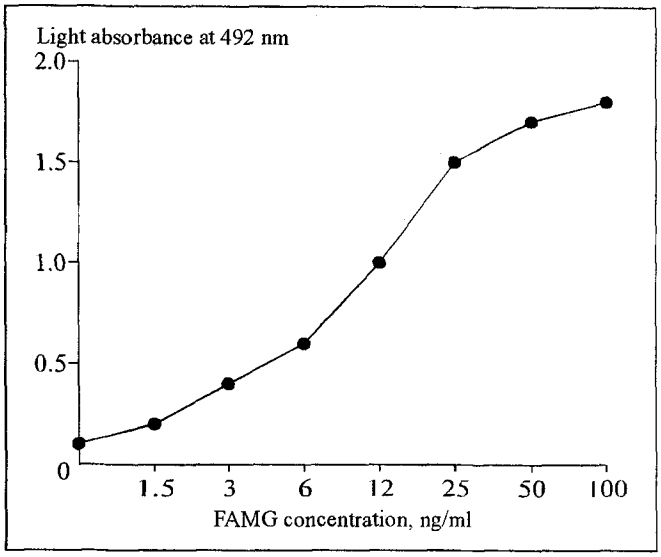

Fig. 1. Standard curve for determination of FAMG content.

On the basis of these MAb we have developed a test-system for quantitative determination of FAMG (FAMG-Fertitest-M) with a sensitivity of 5 ng/ml.

The test-system was analyzed for reproducibility of parameters using calibration curve, the shapes of the curves were compared, and accuracy, reliability, and specificity tests were performed.

Reproducibility of the standard curve. The mean concentration determined on the middle segment of the calibration curve was consistent with the real FAMG concentration, the variation coefficient being equal to 8% (Fig. 1). When FAMG was simultaneously determined in several samples using the control-serum with high, medium, and low FAMG

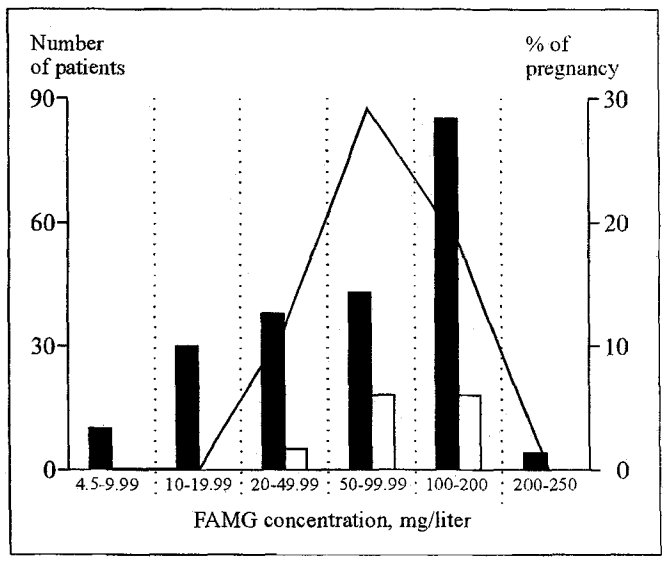

Fig. 2. Successful pregnancy as a function of FAMG concentration in sperm used for extracorporeal fertilization. Shaded bars: unsuccessful attempts; transparent bars: successful attempts. The curve shows the occurrence of pregnancy.

content, the variation coefficient for each concentration was not higher than 9%. When the same samples were analyzed for the FAMG content in our laboratory and in the EKO Research Center, the variation coefficient was <12%.

Reliability control. Variation coefficient in the "recovery test" was <10% and in the "parallelism test" 9%.

Specificity control. The MAb did not cross-react with human serum albumin, bovine serum albumin, trophoblastic β_1-globulin, chorionic gonadotropin, placental α_1-microglobulin, C-reactive protein, and follicle-stimulating and luteinizing hormones.

The test-system was applied at the EKO Center (Moscow) to evaluate the quality of sperm in the treatment of infertility by extracorporeal fertilization. It is known that the *in vitro* fertilizing capacity of morphologically and functionally normal spermatozoa is not always high. Presumably, it depends on the composition of seminal plasma, which contains the major sperm proteins. The relationship between the efficiency of *in vitro* fertilization, pregnancy, and the content of FAMG was analyzed in 245 sperm samples.

FAMG was detected in all the samples, its content varied from 4.5 to 250 mg/liter. The 50-150 mg/liter range of FAMG concentration proved to be optimal for *in vitro* fertilization. The greatest number of pregnancies (17 out of 60 attempts, 28.5%) was recorded after fertilization with sperm with the FAMG content 50-100 mg/liter; the number of pregnancies decreased considerably outside this range (Fig. 2). Pregnancy did not occur when the FAMG content was lower than 22 mg/liter and higher than 200 mg/liter.

Thus, FAMG-Fertitest-M test-system for quantitative determination of FAMG can be used in clinical practice to evaluate the quality of sperm in the treatment of infertility by extracorporeal fertilization.

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