

IMMUNOENZYME ASSAY OF FERTILITY ALPHA -MICROGLOBULIN IN MALE AND FEMALE BLOOD SERUM

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Fertility α_2 -microglobulin (FAMG) was first identified by Petrunin and co-workers in 1976 as an organ-specific placental antigen, secreted into the amniotic fluid [2]. Later, FAMG was found in sperm, the seminal vesicles, menstrual blood, and the endometrium in the secretory phase of the menstrual cycle [3], and for that reason it was called FAMG [4]. It has been shown that FAMG is synthesized by decidual tissue and an enzymic role has been postulated for FAMG in prostglandin synthesis [6].

An antigen immunologically identical with FAMG was subsequently described under the names of "uterine α -globulin" [14], "progesterone-dependent endometrial protein" [11] and, finally, "placental protein 14" [8]. A series of subsequent comparisons proved the immunologic identity of the above-mentioned proteins [8, 12, 15].

Because of the low serum FAMG concentration, it has been necessary to develop highly sensitive methods of analysis for its determination, namely radioimmunoassay and immunoenzyme assay. Radioimmunologic methods of determination of this protein have been developed in recent years in several laboratories [9, 10, 13], but not all proved to be sufficiently sensitive to detect FAMG in the blood serum of nonpregnant women [10].

Since FAMG has definite diagnostic value in obstetrics and gynecology [7], the aim of this investigation was to develop a highly sensitive method of immunoenzyme assay to detect FAMG and determine its normal serum levels in healthy blood donors and pregnant women with a normal course of pregnancy.

EXPERIMENTAL METHOD

Antiserum to RAMG was prepared by immunizing rabbits with a preparation of FAMG isolated from an extract of decidual tissue obtained during abortions. The method of purifying FAMG by adsorption chromatography on silica-gel and salt precipitation was described by the writers

TABLE 1. Immunoenzyme Assay of FIMG in Blood Serum of Healthy Blood Donors and of Women with Normal Pregnancy

Group tested	Number tested	FAMG concentration, ng/ml								
		4-8	8-20	20-40	40-80	80-160	160-320	320-640	640-1280	1280-2560
C I with blood grp:										
M	38	0	38	0	0	0	0	0	0	0
F	16	0	14	2	0	0	0	0	0	0
Co II with blood grp:										
M	63	0	63	0	0	0	0	0	0	0
F	45	0	40	5	0	0	0	0	0	0
C III with blood grp:										
M	28	0	28	0	0	0	0	0	0	0
F	45	0	38	7	0	0	0	0	0	0
C IV with blood grp:										
M	22	0	22	0	0	0	0	0	0	0
F	24	0	20	4	0	0	0	0	0	0
Pregnant women										
I Trimester	56	0	0	0	0	0	0	11	27	18
II Trimester	22	0	0	0	1	11	8	2	0	0
III Trimester	31	0	0	0	6	23	2	0	0	0

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previously [5]. Antibodies to FAMG were isolated from monospecific antiserum with the aid of an antigenic immunosorbent, obtained by immobilizing the FAMG preparation of CNBr-sepharose.

The isolated antibodies, eluted from the immunosorbent with glycine-sulfuric acid buffer, pH 2.4), were used for immobilization on a solid phase (3040-11TM polystyrene plates for tissue culture; Falcon, USA), and for preparation of a conjugate of antibodies with enzyme (type 6 horseradish peroxidase; Sigma, USA). The technique of immunoenzyme assay was described by the writers previously [1].

EXPERIMENTAL RESULTS

The limits of sensitivity of the method of immunoenzyme assay of FAMG, under the conditions used, was 4 ng of antigen in 1 ml of serum. The FAMG concentration in the blood sera of all male blood donors tested was between 8 and 20 ng/ml (Table 1), and accordingly this range could be accepted as "normal." The upper limit of the "normal" serum FAMG level in women was higher (up to 40 ng/ml); all women with FAMG in a concentration of 20-40 ng/ml, moreover, were in the secretory phase of the menstrual cycle. Elevation of the serum FAMG level in the second phase of the menstrual cycle correlates with the time course of the concentration of this protein in the endometrial tissue [3, 10]. In the proliferative phase the serum FAMG level corresponded to that in men.

No correlation could be discovered between the serum FAMG level and the blood group.

With the development of pregnancy there was a sharp rise in the serum FAMG concentration, especially in the first trimester - 1000 ng/ml (Table 1). The level of this protein in the serum then fell considerably, to reach about 150 ng/ml in the second and 100 ng/ml in the third trimester. Other workers, who determined this antigen by radioimmunoassay also found a similar time course of the serum FAMG concentration during the development of pregnancy [10, 13].

FAMG was found to be present in the highest concentration in the decidual tissue in the early stages of pregnancy, and most of it is secreted into the amniotic fluid [2, 7, 10]. It is not yet settled whether FAMG secretion into the blood serum of pregnant women is of physiological importance on its own account or whether it is merely a passive "echo" of its metabolism in the decidual tissue and amniotic fluid.

The normal limits of the FAMG concentration in blood serum established above can be used as a control for estimating the diagnostic value of the test for this protein in pathology.

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