Immunoglobulins IgG, IgA, IgM, complement C3, C4 and ferritin and transferrin levels in serum and follicular fluid in IVF patients

T. MANTZAVINOS - N. DALAMANGA - D. HASSIAKOS - F. DIMITRIADOU
O. GREGORIOU - P. A. ZOURLAS

Summary: Serum and follicular fluid immunoglobulin (IgG, IgA, IgM) and complement (C3, C4), as well as transferrin and ferritin concentrations were measured in 82 consecutive IVF patients (Pregnant: Group A, and non pregnant: Group B). Higher serum concentrations were observed in complement in all Immunoglobulins but IgM which was found to be significantly lower in follicular fluid in both groups. Also there were no statistically significant differences observed for transferrin and ferritin levels in either compartment or among the groups. No correlation was found for the above parameters and the in vitro fertilization outcome in terms of oocytes retrieved, fertilized and embryos implanted.

INTRODUCTION

There is considerable evidence suggesting that the immune system plays an important role in the follicular development and function in patients undergoing ovarian hyperstimulation for the purpose of in vitro fertilization (IVF) ([1, 2]).

The levels of T-Lymphocyte subpopulations (CD8 and CD4) found in the follicular fluid and in the peripheral blood of women in IVF programs have been implicated in local follicular immunoregulation. Furthermore, human chorionic gonadotropin (hCG) used in ovarian stimulation is known to have immunological properties ([3, 4]). Autoantibodies in the follicular fluid appear to play an important role in IVF failure and reports have shown that patients with autoimmune diseases have reduced fertilization and pregnancy rates ([5]).

However, the iron binding proteins transferrin and ferritin are essential for cell proliferation. Transferrin receptors in the actively proliferating cells are necessary and responsible for the intracellular uptake and distribution of iron which represents an essential component of the cytochrome energy production system ([6]). The increase of granulosa cells in coordinating the developmental events associated with follicle maturation in In Vitro Fertilization is a process associated with an increased need for iron.

A close relationship has been observed between follicular maturation rate and follicular transferrin levels along with its

2nd Dept. of Ob-Gyn. of the University of Athens
Areteion Hospital, 76 V. Sofias Av.,
Athens 11528, Greece
Euromedica Medical Diagnostic Institute
of High Technology,
2-4 Mesogion Av., Athens 11527, Greece

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receptors in granulosa cells. Satisfactory follicular transferrin levels have also been found in the follicle whose oocyte participated in successful IVF outcome ([7]).

The purpose of the study has been the assessment of the immune status of the follicular fluid and peripheral blood in patients enrolled in our IVF program by measuring the immunoglobulin (IgG, IgA, IgM) and complement (C3, C4) levels as well as the assessment of the iron binding proteins by measuring the corresponding transferrin and ferritin levels.

MATERIALS AND METHODS

Eighty-two consecutive patients who presented themselves at our institute for IVF served as the study population. Patients with family history of autoimmune disease and history of endometriosis were not included in the study.

Controlled ovarian hyperstimulation was accomplished with human menopausal gonadotropin (hMG) and follicle stimulating hormone (FSH), (hMG$\pm$FSH) beginning on cycle day 4, in conjunction with pituitary desensitization using a GnRH analog (Suprefact nasal spray, Hoechst, Greece), beginning on cycle day 2. Blood was drawn daily starting on day 3, for determination of serum estradiol (E_2) by radioimmunoassay. Beginning on day 6, follicle development was monitored by transvaginal ultrasonography. Human chorionic gonadotropin (10,000 IU) was administered intramuscularly (IM) when sonography revealed at least two follicles measuring $\geq$16 mm in diameter, taking into consideration the serum estradiol levels and clinical parameters. Oocyte retrieval was performed 34 hours later by ultrasound- guided transvaginal aspiration. All follicular fluids after the oocytes had been removed were immediately frozen at $-80°$ C. The serum from peripheral blood was also preserved in the same way. Morphologic classification of embryos was done just before embryo transfer using established criteria ([8]).

The immunoglobulin IgG, IgA, IgM, the C3 and C4 fraction of complement as well as follicular and serum transferrin concentrations were determined using a radial immunodiffusion technique ([9]). An immunoenzymatic method was used for measuring ferritin concentrations ([10]).

The statistical analysis was performed using the student's t-test.

RESULTS

For the statistical analysis 19 patients with sperm abnormalities were excluded from the study. From the remaining patients, 15 conceived (14 clinical and 1 biochemical pregnancy, group A) and 48 failed to do so (group B).

The patient population and their IVF outcome are shown in Table 1. There were no statistically significant differences found for the patients ages and mean peak E_2 levels between the two groups. On the contrary, patients who conceived had greater statistically significant number of oocyte retrieved, fertilized and number of embryos transferred ($p<0.01$, <0.01 and <0.05, respectively). The implanta-

Table 1. — *IVF outcome in all patients. group (pregnant) and B (non-pregnant).*

	A	B	p
No. of patients	15	48	
Mean age (years)	33.8±5.1	33.8±4.1	NS
E2 levels (pg/ml) (Mean ±SD)	4951.9 ±3479.0	3618.5 ±2553.7	NS
Oocytes retrieved (Mean ±SD/pt)	222 14.8±10.2	437 9.0±5.4	<0.01
Oocytes fertilized (Mean ±SD/pt)	159 (71.6%) 10.6±6.4	286 (65.4%) 6.0±4.3	<0.01
Embryos transferred (Mean ±SD/pt)	69 4.6±1.5	151 3.6±1.6	<0.05
Pregnancies	15 *		

(*) 1 biochemical.

Table 2. — *Grading of embryos before transfer.*

	Group A (N_o = 69)	Group B (N_o = 157)
Grade 1	49%	10%
Grade 2	25%	38%
Grade 3	14%	19%
Grade 4	12%	33%

Table 3. — *Immunoglobulin levels (mean ±SD) in serum and follicular fluid (mg/dl).*

	IgG		IgA		IgM	
	A	B	A	B	A	B
Serum	1182.2 ±331.9	1027.3 ±335.3	126.8 ±75.9	142.4 ±67.8	155.7 ±56.7	187.9 ±85.0
F. Fluid	580.3 ±199.7	553.1 ±279.8	60.1 ±48.1	41.3 ±20.1	1.5 ±0.7	10.4 ±10.7

$P>0.05$, except for IgA in follicular fluid. ($p<0.02$).

tion rate per embryo transferred in this group of patients was 8.63%. The grading of embryos is shown in Table 2.

The mean ±SD serum and follicular immunoglobulin levels are presented in Table 3 and depicted in Fig. 1. There were no statistically significant differences found between the two groups in either compartment, except for the IgA of the follicular fluid which was slightly increased in group A ($p<0.05$). Lower immunoglobulin levels were found in the follicular fluid in comparison to those observed in the peripheral blood. This was strongly manifested for the follicular IgM levels which were 90% and 99.5% lower than those of serum, in group A and B, respectively.

The mean ±SD levels of the complement compounds C3 and C4 in serum and follicular fluid in both groups are presented in Table 4 and depicted in Fig. 1. There were no statistically significant dif-

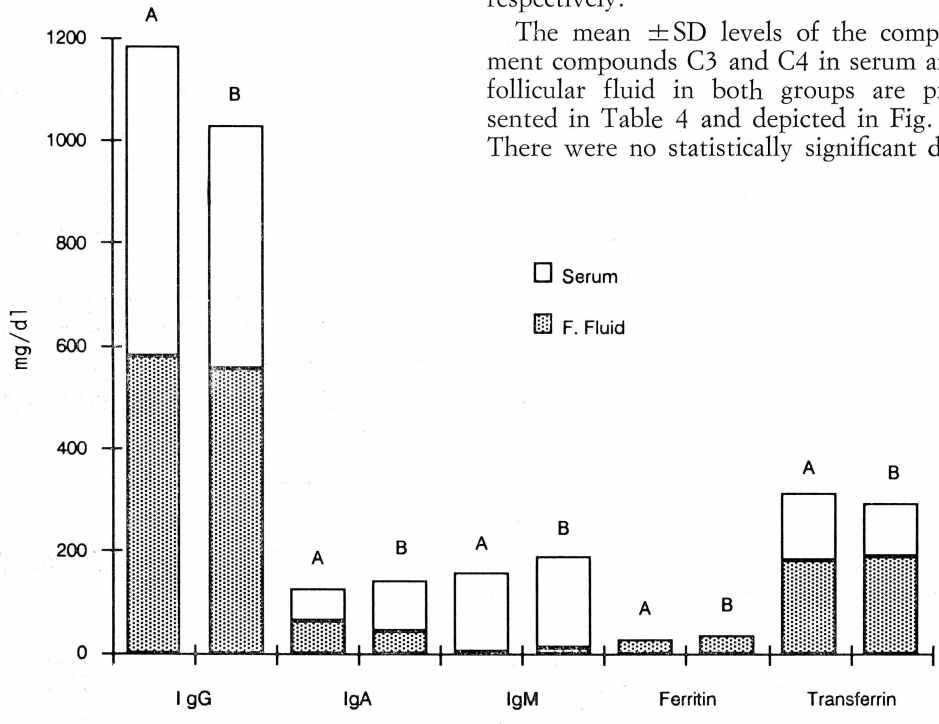

Fig. 1. — Immunoglobulins IgG, IgA, IgM, Complement C3, C4 and Ferritin and Transferrin levels in serum and follicular fluid in all patients (A: Pregnant, B: Non pregnant).

Table 4. — *Complement (C3 and C4) levels (mean ±SD) in serum and follicular fluid (mg/dl).*

	C3		C4	
	A	B	A	B
Serum	115.8 ±42.7	99.5 ±32.4	21.2 ±8.9	26.4 ±9.3
F. Fluid	54.3 ±30.4	58.4 ±29.1	11.5 ±7.2	18.1 ±8.8

P>0.05, except for C4 in follicular fluid, (p<0.02).

ferences found between the two groups in blood and follicular fluid except for the lower C4 levels noticed in the follicular fluid of patients who conceived (p<0.02). The C3 and C4 follicular fluid levels were 32%-53% of the corresponding levels found in peripheral blood.

Table 5 presents the mean ±SD ferritin and transferrin levels observed in blood and follicular fluid. There were no statistically significant differences for these two proteins in either study group and in either compartment (p>0.05). Slightly higher ferritin levels were present in serum in comparison with follicular fluid in both groups (24.6±17.9 and 24.9±14.2 mg/dl in group A, and 29.1±18.34 and 34.32±20.3 mg/dl in group B, respectively). On the contrary, serum transferrin levels were increased in relation to those in follicular fluid for both groups (310.8 ±51.9 and 179.4±62.1 mg/dl in group A, and 291.0±66.4 and 187.5±59.24 mg/dl in group B, respectively). Follicular transferrin levels varied at the levels of 36% and 43% of the corresponding serum levels in group A and B respectively. Serum and follicular ferritin and transferrin levels are depicted in Fig. 1.

DISCUSSION

There is no general agreement concerning the impact of the circulating antibodies and the in vitro fertilization outcome. Earlier reports have shown that immunoglobulin IgG, IgA and complement compounds C3 and C4 concentrations in follicular fluid are similar to those observed in serum. On the contrary, IgM was found to be significantly lower in follicular fluid, suggesting a specific blood - follicle barrier for IgM ([5, 11]).

In our study there were lower follicular levels for all immunoglobulins (IgG, IgA, IgM) and for the complement C3 and C4 in comparison to those of serum levels in both study groups. The low IgM follicular levels noticed in both groups seem to suggest the existence of a specific IgM blood-follicle barrier. The immunoglobulins and complementary follicular and serum levels did not show any correlation with the in vitro fertilization outcome (oocytes retrieved, fertilized and embryos implanted).

Previously reported data showed that the follicular transferrin concentrations were directly related to a successful oocyte maturation and fertilization in IVF ([7]). In our study both transferrin and ferritin failed to establish this observation since their serum and follicular fluid levels in both study groups had no statistical cor-

Table 5. — *Ferritin and transferrin levels (mean* ±SD) in serum and follicular fluid (mg/dl).

	Ferritin		Transferrin	
	A	B	A	B
Serum	24.6±17.9	29.2±18.3	310.8±51.8	291.0±66.4
F. Fluid	24.9±14.2	34.3±20.3	179.4±62.1	187.5±59.2

P>0.05

relation. The increased follicular fluid transferrin and ferritin levels observed were in agreement with previous studies but differed in that in our study the follicular transferrin levels were lower than those observed in serum ([12]).

The fact is that granulosa cells of the maturating follicle, actively proliferating for the oocyte maturation process and the capillary permeability of the vascular network of the theca, play an active role in extraintracellular iron transport. There have been speculations about the contribution of granulosa cells in transferrin synthesis. Studies related to transferrin receptors in granulosa cells showed that transferrin seems to the be of extracellular origin, while the gonadotropin or non-ovarian stimulation does not affect those receptors ([7, 12]).

In conclusion, although the immune system is the basic regulator of life as a the knowledge of the immune status in the peripheral and in the local ovarian environment at present does not offer enough confirmation, and further investigation in the future might be helpful in predicting the success or failure of in vitro fertilization. In addition the increased ferritin and transferrin concentrations observed in this study show their essential role in follicular maturation but their actual overall contribution for a successful IVF outcome needs further evaluation.

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Address reprint requests to:
T. MANTZAVINOS
6 Gennadiou St.
Athens 11521, (Greece)