# Assessment of autoantibodies to the zona pellucida in serum and follicular fluid in in-vitro fertilization patients

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*Summary:* Eighty two consecutive IVF patients enrolled in our In-Vitro Fertilization program were investigated on the presence of anti-zona pellucida antibodies in the peripheral blood and follicular fluid, using a haemaglutination test (ovarian zona pellucida HAT) and a zona pellucida slide test, respectively. Overall higher proportion of antibody activity was observed in pregnant patients in both compartments (60% and 53%) in serum and follicular fluid, respectively) than the activity observed in non-pregnant (25% and 48%, respectively). There were no statistically significant differences found for the mean number of oocytes retrieved and fertilized irrespective of the presence of antibodies in serum or in follicular fluid, each one being taken into account separately. However, higher fertilization rates were observed in serum or follicular fluid antibody negative patients (68.2% and 71.2%, respectively) than those who were tested positive (36.6% and 65.4%, respectively). The same was true for the presence of anti-zona pellucida antibodies in serum and in follicular fluid, taking into consideration their presence or absence in both compartments simultaneously.

*Key words*: In-vitro fertilization; Zona pellucida antibodies.

## INTRODUCTION

The ovary and specially the zona pellucida appears to be a good source of ovarian antigens strong and in large quantities [1].

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The breakdown and absorption of the zona in the ovary and reproductive tract during the reproductive life could continually expose the zona artigen(s) to the immune system and thus result in autosensitization [1, 2]. Attempts have been made to investigate the immunological effects of antibodies to zona pellucida antigens on the reproductive process. These autoantibodies alter the zona pellucida surface in such a way that receptor sites are no longer available to spermatozoa so that attachment, penetration and consequent fertilization are inhibited. Implantation of fertilized eggs is also inhibited by preventing the embryo from escaping the zona [3].

The possible causal relationship between the presence of autoantibodies to zona pellucida and human infertility and the potential role of zona pellucida antigens as targets for immunocontraception are under way. However, conflicting data exist today concerning the etiologic significance of the zona pellucida antoantibodies in the development of infertility [4-6].

The present investigation aims to assess the zona pellucida autoantibodies in serum and in follicular fluid in women who have undergone ovarian hyperstimulation for in vitro fertilization (IVF) in our institute and to evaluate their significance in the fertilization process.

## MATERIALS AND METHODS

The study population consisted of eighty-two consecutive IVF patients who underwent ovarian hyperstimulation in their first attempt. Patients with family history of autoimmune disease and history of endometriosis were excluded from the study.

Ovarian superovulation was induced with human menopausal gonadotropin (h-MG) and follicle stumulating hormone (FSH), (hMG±FSH) beginning on cycle day 4 after pituitary down regulation with buserelin acetate (Suprefact, Hoechst, Greece), given intranasally from cycle day 2. Determination of serum estradiol ($E_2$) started on day 3, and 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 ≥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. Morphologic classification of embryos was done just before embryo transfer using established criteria [7].

All follicular fluids after the oocytes had been removed were immediately frozen at −20° C. The serum from peripheral blood was also preserved in the same way. All samples were inactivated by heating at 56° C for 30 minutes prior to testing. The detection and estimation of titre of autoantibodies in serum to the ovarian zona pellucida was performed by using a haemagglutination test (ovarian zona pellucida HAT) and of those in the follicular fluid by using a zona pellucida slide test [8, 9].

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 pregnancies - 1 biochemical, group A) and 48 failed to do so (group B).

The patient population and their IVF outcome are shown in Table 1. There were statistically significant differences found for the patients ages and mean peak $E_2$ levels between the two groups. On the other hand, patients who conceived had greater statistically significant number of oocytes retrieved, fertilized and number of embrios transferred ($p<0.01$, $<0.01$ and $<0.05$, respectively). The implantation rate per embryo transferred was 8.63%.

Table 2 presents analytically the intensity of anti-zona pellucida antibody reaction observed in follicular fluid and serum in both groups. Overall, a higher proportion of antibody activity was observed in pregnant patients in both compartments (60% and 53% in serum and

Table 1. — *IVF results in group A (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 |
| $E_2$ 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. — *Incidence of anti-zona pellucida antibody activity in serum and follicular fluid.*

| | | Intensity of anti-zona reaction | | | | |
|---|---|---|---|---|---|---|
| | | + | ++ | +++ | — | Total (+, ++, +++) |
| SERUM | Pregnant | 3 | 1 | 5 | 6 (40%) | 9 (60%) |
| | Non - Pregnant | 22 | 5 | 9 | 36 (75%) | 12 (25%) |
| F. FLUID | Pregnant | 3 | 1 | 3 | 7 (47%) | 8 (53%) |
| | Non - Pregnant | 10 | 6 | 9 | 25 (52%) | 23 (48%) |

follicular fluid, respectively). The corresponding numbers in non-pregnant patients were 25% and 48%, respectively. Depiction of the above findings is shown in figure 1. There were no statistically significant differences for the mean age and type of infertility for patients tested positive or negative for anti-zona pellucida antibody in serum and/or in follicular fluid.

Tables 3 and 4 present details of the anti-zona pellucida antibodies and the number of oocyte retrieved and fertilized in patients positive or negative for antibodies in serum or in follicular fluid. There were no statistically significant dif-

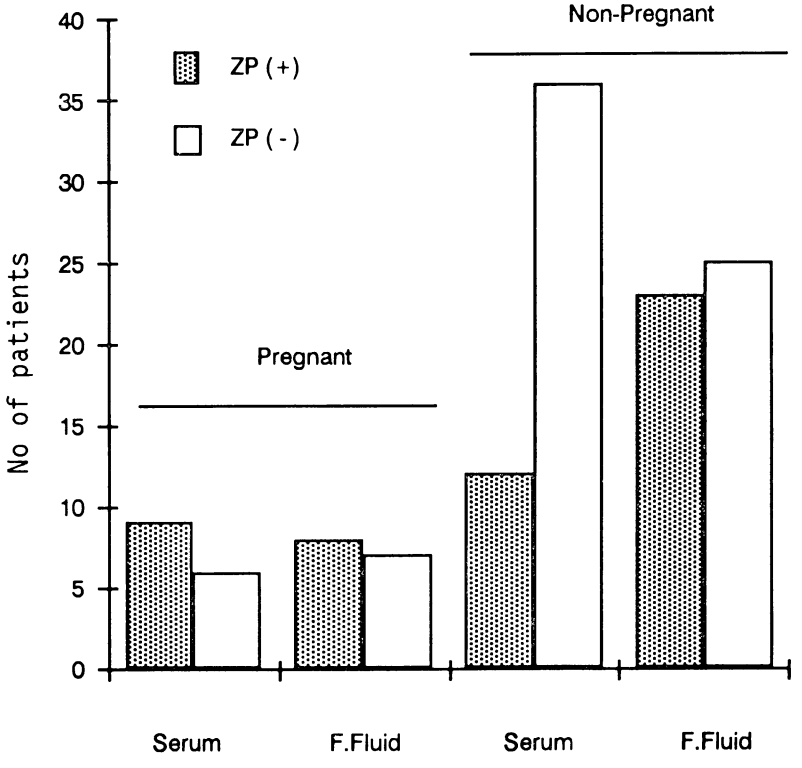


Fig. 1. — Autoantibodies to zona pellucida (ZP) in serum and follicular fluid in all IVF patients.

Table 3. — *Anti-zona pellucida antibody activity and fertilization rates.*

| | Serum | | F. Fluid | |
|---|---|---|---|---|
| | + | − | + | − |
| No of patients | 45 | 18 | 31 | 32 |
| No of oocytes retrieved (Mean ±SD/pt) | 411 (9.4±6.3) | 198 (11.0±7.1) | 303 (9.8±6.9) | 343 (10.9±7.6) |
| No of oocytes fertilized | 269 | 141 (71.2%) | 200 (36.6%) | 234 (68.2%) |
| (Mean ±/pt) | (5.5±3.6) | (7.8±5.6) | (6.5±4.9) | (7.5±5.5) |

$p>0.05$

ferences found for the mean number of oocytes retrieved and fertilized irrespective of the presence of antibodies in serum or in follicular fluid, each being taken into account separately. Although higher fertilization rates were observed in serum or follicular fluid antibody negative patients (68.2% and 71.2%, respectively) than those who were tested positive (36.6% and 65.4%, respectively).

The same was also true for the presence of anti-zona pellucida antibodies in serum and in follicular fluid taken into consideration their presence or absence in both compartments simultaneously (Table 4). The fertilization rates observed were higher in antibody negative patients than in those found to be positive (71.2% and 66.1%, respectively).

Table 4. — *Anti-zona pellucida antibody acidity and fertilization rates.*

| | Serum - F. Fluid (+) | Serum - F. Fluid (−) |
|---|---|---|
| No of patients | 30 | 16 |
| No of oocytes retrieved (Mean ±SD/pt) | 295 (9.8±7.0) | 188 (1.8±7.1) |
| No of oocytes fertilized (Mean ±SD/pt) | 195 (66.1%) (6.5±4.9%) | 134 (71.2%) (8.4±5.7) |

$p>0.05$

## DISCUSSION

Although IVF reduces the influence of immunological mechanisms that have been responsible for subfertility, the fertilization failure, excluding obvious causal factors, still remains obscure ([2]).

Ovarian antibodies occurring naturally due to exposure of the immune system to atretic oocytes in the ovary or degenerative oocytes in the reproductive tract, have been indicated as a cause of subfertility and of premature menopause. An incidence of 15-76% of ovarian antibodies has been reported in subfertile women. However, positive titres do not always prevent fertilization and pregnancies, and their significance, as well as treatment with immunosuppression, remains controversial ([2, 10, 11]).

Antizona pellucida antibodies have been the most widely investigated, and atresia of multiple oocytes in immature follicles during superovulation for IVF may be the causing factor of the high antibody titres found in women who have previously had superovulation therapy ([12]). Failure of adequate gonadotropin stimulation has also resulted in increased ovarian antibody titres ([13]).

The results of the present study indicate surprisingly enough that anti-zona pellucida antibodies are present in serum and follicular fluid in a significant number of IVF patients. In our study the patient's

advanced age (mean 33.8±5.1 years) might have been partly responsible. According to previous reports the repeated exposure of the immune system to the zona for several years through egg atresia and absorption of ovulated eggs in the reproductive tract may account for the production of antibodies to the zona ([1]).

Unexpectedly, a higher proportion of antibody positive samples (follicular fluid or serum) were observed in non-pregnant patients in comparison to the pregnant ones, but this did not effect the fertilization rate in either group. The majority of the investigators however observed (for non-IVF patients) the fertile patients to be negative for anti-zona antibody or to be in lower proportion compared to infertile ones ([5, 14]). There were also no effects on fertilization rates from oocytes retrieved from patients tested positive in follicular fluid or serum, separately or combined.

In conclusion, the findings observed in our study further implicate the potential role of the anti-zona pellucida antibodies in IVF patients. The fact that there was no correlation and effect demonstrated on fertilization for all groups and subgroups of patients suggests that their actual role is obscure and further investigation is needed.

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