

Effect of Anti Phospholipid Antibodies on in vitro fertilization/intracytoplasmic sperm injection outcome

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Abstract

Objective: The study aimed to determine the relationship between presence of antiphospholipid antibodies (APLs) and clinical pregnancy rate in patients undergoing IVF/ICSI procedures.

Materials and methods: This descriptive-analytic study performed on two hundred consecutive women referred for IVF/ICSI in Vali-e-Asr Reproductive Health Research Center. Serum levels of APLs , anticardiolipin [aCL], antiphosphatidic acid [aPA], antiphosphatidyl choline [aPC] and antiphosphatidylserine [aPS] were checked for all patients before starting IVF cycles. APLs seropositivity and clinical pregnancy rate were determined. T-test and Mann-Whitney were used to compare two groups. P value <0.05 was considered significant.

Results: 23 women (11.5%) were APL positive. Twenty nine women of 177 APL seronegative patients (16.4%) became pregnant while only one of 23 seropositive patients (4.3%) was pregnant. Clinical pregnancy rate was not significantly different in two groups.

Conclusion: Although APLs were common, these antibodies did not affect the outcome of IVF/ICSI procedures. Thus screening for APLs is not recommended in women undergoing these procedures.

Key words: *Anti phospholipid antibodies, pregnancy rate, In Vitro Fertilization, Intra Cytoplasmic Sperm Injection*

Introduction

Antiphospholipid antibodies are a group of antibodies that react with negatively charged phospholipids. These antibodies include anticardiolipin antibody; lupus anticoagulant; antibodies

to phosphatidylserine; phosphatidylinositol and phosphatidylethanolamine and antibodies to the cofactor β_2 glycoprotein I (a β_2 glycoprotein) (1). Antiphospholipid syndrome (APS) has been defined as a condition which history of recurrent pregnancy loss and or thrombosis is accompanied by persistently positive tests for APA (2). Several classes of APLs have been described. The presence of these antibodies, coupled with the clinical manifestations of recurrent miscarriage, arterial or venous thrombosis, or thrombocytopenia in the presence of

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overt autoimmune disease, has been termed "Primary Antiphospholipid Syndrome" (3). The importance of this syndrome as an etiologic factor in some cases of recurrent miscarriage is now well established, as is the efficacy of thromboprophylactic treatment with low-dose aspirin and heparin in this group of women (4, 5).

In recent years, one of the greatest controversies in assisted reproduction has been whether the presence of antiphospholipid antibodies (APLs) in the serum of the female partner negatively influences the outcome of IVF/ICSI. Many studies have examined this purported association with variable conclusions (6-24). This question has clinical relevance. The success of aspirin and heparin in the treatment of APL-recurrent miscarriage (4,5) together with the few reports suggesting a possible role for APLs in the failure of IVF/ICSI has led to speculation about whether treatment with aspirin and heparin can improve pregnancy rates in women undergoing IVF/ICSI who have circulating APLs. For example two investigators have proposed aspirin, IVIG and heparin as treatment that might increase the likelihood of implantation in patients seropositive for APL (6, 21). In an other study the use of heparin and aspirin have not increased the success of IVF in patients with APLs (1).

The aims of this study were to determine the prevalence of APLs in women referred for IVF/ICSI and to compare the early outcomes of pregnancy in APL seropositive patients in comparison to APL seronegative patients.

Material and methods

Two hundred women referred to Vali- e-Asr Reproductive Health Research Center for IVF/ICSI between April 2002 and March 2003 were recruited to the study. Exclusion criteria were history of

autoimmune diseases, history of recurrent miscarriages or clinical thrombosis and patients' dissatisfaction. All patients underwent controlled ovarian hyperstimulation (COH) with GnRH agonists and hMG. Before starting IVF/ICSI cycles, blood samples were taken to determine the presence of aCL, aPC, aPS and aPA antibodies. All blood samples were immediately tested for the presence of both IgG and IgM serotypes of aforementioned autoantibodies by standardized enzyme immunoassay (EIA). Values more than 10 U/ml were considered positive.

Early outcomes of pregnancy including the rates of clinical pregnancy (documented by detection of fetal heart activity by ultrasonography two weeks after positive pregnancy test), chemical pregnancy (detection of β -hCG in woman's serum), blighted ovum (detection of large gestational sac without fetal pole) and multiple pregnancy were compared between two groups. Serum β -hCG measurements were performed two to three weeks after oocyte retrieval.

After collecting the data, SPSS 11 software was used for statistical analysis of this cross sectional descriptive analytic study. T-test and Mann-Whitney were used to compare two groups. Fisher's exact test was used for other comparisons between APL-positive and APL-negative patients. P-value <0.05 was considered significant.

Results

Two hundred patients were studied in this study with the mean age of 28.9 years; ranging 18-48. Totally 23 patients (11.5%) were seropositive for at least one of APL antibodies. All the 23 patients were aCL antibody positive (IgG or IgM). The mean age of patients was not significantly different in

seropositive and seronegative groups (28 years and 29 years respectively).

Duration of infertility ranged from 1- 21 years with a mean of 6.7 years in studied women. Duration of infertility was not significantly different between two studied groups (6.4 versus 6.8 years).

The prevalence of APL seropositivity was not significantly different between the groups of patients with primary and secondary infertility (10.6% and 15.4% respectively).

With respect to the number of previous IVF/ICSI cycles, patients were divided into 2 groups: patients with 3 or less (181 patients or 90.5%) and patients with more than 3 times of previous cycles, i.e. recurrent IVF/ICSI failure (19 patients or 9.5%). The prevalence of APL antibodies in these two groups was 11% and 15.8%,

respectively with no significant difference.

Of 200 women, gestational sac was detected in 32 patients (16%). In 30 patients (15%) fetal heart activity was detected by ultrasonography. Only two cases (1%) of blighted ovum pregnancies, occurred in seronegative patients. The rates of clinical pregnancy and multiple pregnancy were not different in seropositive and seronegative groups (table 1).

Also there was no significant difference in clinical pregnancy rates between seropositive and seronegative groups with respect to age, number of previous IVF/ICSI cycles, type of infertility, duration of infertility and cause of infertility.

Table 1: IVF/ICSI outcome in APL positive and negative patients

Outcome	APLs positive (n=23)	APLs negative (n=177)	p-value
Chemical pregnancy	3 (13%)	31 (17.5%)	NS
Clinical pregnancy	1 (4.3%)	29 (16.4%)	NS
Blighted ovum	0	2 (1.1%)	NS
Multiple pregnancy	0	4 (2.3%)	NS

Discussion

The role of APL antibodies in infertility and recurrent pregnancy loss has been the focus of several clinical reports (1-5). In recent years, this association has led some investigators to speculate that APL antibodies may also be associated with IVF failure (6- 11). The results of such studies are very much conflicting and this question has been one of the greatest controversies in assisted reproduction.

In our study, the prevalence of APLs in infertile women undergoing IVF or ICSI procedures was 11.5%. This was higher

than the prevalence of APL antibodies in low-risk population (25, 26). This high prevalence can not be attributed to COH regimen because samples were taken before initiation of cycles. In previous similar studies, the prevalence of APL antibodies varies from 15% to 53% (15, 21) which is attributed to the lack of standardization in APL laboratory testing. In our patient population, the prevalence of APLs was lower than other similar studies which can be due to the different demographic characteristics of patients under study, lack of standardization in antibody testing and missing the measurement of some

measurable antibodies because of our limitations.

This study has demonstrated that despite of the high prevalence of APL antibodies in women referred for IVF/ICSI, these antibodies do not affect the outcome of IVF/ICSI procedures. It is suggested that routine screening of women undergoing IVF/ICSI for the presence of APL antibodies is not recommended.

The role of APLs as a cause of infertility is uncertain, though, a group of candidates for infertility treatment are APL seropositive simultaneously. Regardless of the cause and effect relation, APLs are discussed in different aspects of infertility as below.

Udoff LC, et al considered that the medications used for ovulation induction cause an increase in ovarian estrogen production beyond that typical of a normal menstrual cycle and suggested clinicians to be appropriately concerned about the potential adverse effects of this estrogen surge on the clinical status of women with autoimmune disease (27). For APS, a primary concern would be that of thrombosis or embolism (27).

Houg DL and et al showed that the presence of antiphospholipid antibodies increases the thrombotic risk related to ovulation –induction therapy especially in systemic Lupus erythematosus patients (28). In other studies APL were shown to be significantly associated with late pregnancy losses (29, 30).

A group of studies present women with endometriosis marking antiphospholipid antibodies more often than women without endometriosis (31)

In conclusion apart of our finding in present study which denies APL screening in all IVF patients, considering other systemic effects of APLs it is justified to perform APLs evaluation in the group of indicated patients.

Although APLs were common, these antibodies did not affect the outcome of IVF/ICSI procedures. Thus screening for APLs is not routinely recommended in

all women undergoing IVF/ICSI and its performance should be considered in only indicated cases.

References

- 1- Stern C , Chamley L , Norris H, Hale L , Gordon Baker HW.A randomized , double –blind, placebo-controlled trial of heparin and aspirin for women with in vitro fertilization implantation failure and antiphospholipid or antinuclear antibodies. *Fertility and Sterility* 2003; 80 (2): 376-383.
- 2- Mwanda OW. Lupus anticoagulants: pathophysiology, clinical and laboratory associations: a review. *East Afr Med J* 2003; 80(11): 564-568.
- 3- Harris EN. Clinical and serological features of the antiphospholipid syndrom (APS). *Br J Rheumatol* 1987; 26: 19.
- 4- Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am J Obstet Gynecol* 1996; 174: 1584-1589.
- 5- Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *BMJ* 1997; 314: 253-257.
- 6- Balasch J, Creus M, Fabregues F, Font J, Martorell J, Vanrell JA. Intravenous immunoglobulin preceding in vitro fertilization-embryo transfer for patients with repeated failure of embryo transfer. *Fertil Steril* 1996; 65: 655-658.
- 7- Birkenfeld A, Mukaida T, Minichiello L, Jackson M, Kase NG, Yemini M. Incidence of autoimmune antibodies in failed embryo transfer cycles. *Am J Reprod Immunol* 1994; 31: 65-68.
- 8- Coulam CB, Krysa LW, Bustillo M. Intravenous immunoglobulin for in-vitro fertilization failure. *Hum Reprod* 1994; 9: 2265-2269.
- 9- Balasch J, Creus M, Fabregues F, Reverter JC, Carmona F, Tassies D, et al. Antiphospholipid antibodies and human reproductive failure. *Hum Reprod* 1996; 11: 2310-2315.
- 10-Coulam CB, Kaider BD, Kaider AS, Janowicz P, Roussev RG. Antiphospholipid antibodies associated

- with implantation failure after IVF/ET. *J Assist Reprod Genet* 1997; 14: 603-608.
- 11-Geva E, Yaron Y, Lessing JB, Yovel I, Vardinon N, Burke M, et al. Circulating autoimmune antibodies may be responsible for implantation failure in in-vitro fertilization. *Fertil Steril* 1994; 62: 802-806.
- 12-Bronson R. Immunology and reproductive medicine. *Hum Reprod* 1995; 10: 755-757.
- 13-Coulam CB. Implantation failure and immunotherapy. *Hum Reprod* 1995; 10: 1338-1340.
- 14-Fisch B, Rikover Y, Shohat L, Zurgil N, Tadir Y, Ovadia J, et al. The relationship between in vitro fertilization and naturally occurring antibodies: evidence for increased production of antiphospholipid autoantibodies. *Fertil Steril* 1991; 56: 718-724.
- 15-Birdsall MA, Lockwood GM, Ledger WL, Johnson PM, Chamley LW. Antiphospholipid antibodies in women having in-vitro fertilization. *Hum Reprod* 1996; 11: 1185-1189.
- 16-Denis AL, Guido M, Adler RD, Bergh PA, Brenner C, Scott RT. Antiphospholipid antibodies and pregnancy rates and outcome in in-vitro fertilization patients. *Fertil Steril* 1997; 1084-1090.
- 17-El-Roeiy A, Gleicher N, Fribert J, Confino E, Dudkiewicz A. Correlation between peripheral blood and follicular fluid autoantibodies and impact on in-vitro fertilization. *Obstet Gynecol* 1987; 70: 163-170.
- 18-Gleicher N, Liu H-C, Dudkiewicz A, Rosenwaks Z, Kaberlein G, Pratt D, et al. Autoantibody profiles and immunoglobulin levels as predictors of in vitro fertilization success. *Am J Obstet Gynecol* 1994; 170: 1145-1149.
- 19-Kowalik A, Vichnin M, Liu H-C, Braunch W, Berkeley AS. Midfollicular anticardiolipin and antiphosphatidylserine antibody titers do not correlate with in vitro fertilization outcome. *Fertil Steril* 1997; 68: 298-304.
- 20-Kutteh WH, Yetman DL, Chantilis SJ, Crain J. Effect of antiphospholipid antibodies in women undergoing in-vitro fertilization: role of heparin and aspirin. *Hum Reprod* 1997; 12: 1171-1175.
- 21-Sher G, Feinman M, Zouves C, Kuttner G, Maassarani G, Salem R, et al. High fecundity rates following in-vitro fertilization and embryo transfer in antiphospholipid antibody seropositive women treated with heparin and aspirin. *Hum Reprod* 1994; 9: 2278-2283.
- 22-Hornstein MD, Davis OK, Massey JB, Paulson RJ, Collins JA. Antiphospholipid antibodies and in vitro fertilization success: a meta-analysis. *Fertil Steril* 2000 73(2): 330-333.
- 23-Matsubayashi M, Sugi T, Arai T, Kondo A, Suzuki T, Izumi SI. Different antiphospholipid antibody specificities are found in association with early repeated pregnancy loss versus recurrent in-vitro fertilization failure patients. *Am J Reprod Immunol* 2001; 46(5): 323.
- 24-Backos M, Rai R, Regan L. Antiphospholipid antibody and infertility. *Hum Fertil* 2002; 5(1): 30-34.
- 25-Lockwood CJ, Romero R, Feinberg RF, Clyne LP, Coster B, Hobbins JC. The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. *Am J Obstet Gynecol* 1989; 161: 369-373.
- 26-Pattison NS, Chamley LW, McKay EJ, Liggins GC, Butler WS. Antiphospholipid antibodies in pregnancy: prevalence and clinical association. *Br J Obstet Gynecol* 1993; 100: 909-913.
- 27-Udoff LC, Branch DW. Management of patients with antiphospholipid antibodies undergoing in Vitro fertilization. *J Autoimmune* 2000; 15(2): 209-211.
- 28-Huong DL, Wechsler B, Piette JC, Arfi S, Gallinari C, Darbois Y, et al. Risks of ovulation-induction therapy in systemic Lupus erythematosus. *Br J Rheumatol* 1996; 35(11): 1184-1186.
- 29-Shoenfeld Y, Carp HJ, Molina V, Blank M, Cervera R, Balasch J, et al. Autoantibodies and Prediction of reproductive failure. *Am J Reprod Immunol* 2006; 56(5-6): 337-344.
- 30-Egbase PE, Al Shahrani M, Diejomaoh M, Grudzinskas JG. Antiphospholipid antibodies in infertile couple with two consecutive miscarriages after in-vitro fertilization and embryo transfer. *Hum Reprod* 1999; 14(6): 1483-1486.
- 31-Szymanski W, Zielinska I. Application of IVF in women with endometriosis. *Ginecol Pol* 2001; 72(5): 273-277.