

# A RATIONAL BASIS FOR THE USE OF COMBINED HEPARIN/ASPIRIN AND IVIg IMMUNOTHERAPY IN THE TREATMENT OF RECURRENT IVF FAILURE ASSOCIATED WITH ANTIPHOSPHOLIPID ANTIBODIES

Sher Geoffrey, M.D. \* ‡, Zouves Christo, M.D., Feinman, Michael, MD,\* Maassaran Ghanima, Dr.Med. \*, Matzner William, M.D. §, Chong Penny, M.D. § and Ching Wendell, M.D. §||

\* Pacific Fertility Medical Centers of California

† Harbor UCLA Medical Center

‡ University of Nevada, School of Medicine

§ Reproductive Immunology Associates

|| Sepulveda VA Medical Center / UCLA

## ABSTRACT

**PROBLEMS:** 1). Does the administration of Heparin and Aspirin (H/A) in combination with intravenous Immunoglobulin G (IVIg) improve IVF implantation and birthrates in patients with recurrent IVF failures. 2). Is the effect of such treatment related to the APA status of the patients concerned.

**METHODS:** Subjects consisted of 89 women less than 36 years of age whose infertility was due to causes other than male infertility and who had experienced four or more failed IVF/ET procedures. Fifty two (52) women were APA+ (Group A) and 37 were APA- (Group B). All patients, regardless of their APA status, received H/A (5000 U sq bid), aspirin (81 mg po qd) from the inception of menotropin therapy along with IVIg (20 gm) through a single infusion 3-10 days prior to egg retrieval.

**RESULTS:** Twenty two (22) of Group A (42%) and 7 (19%) of Group B patients achieved live births. (P 0.020).

**CONCLUSIONS:** IVF outcome is significantly improved when H/A & IVIg is administered to APA+ women with repeat IVF failures. APA- women do not appear to benefit from such treatment.

**Key Words:** Antiphospholipid antibodies (APA), Intravenous immunoglobulin G (IVIg), heparin/aspirin (H/A), in vitro fertilization and embryo (IVF/ET).

Reprint requests should be addressed to: Geoffrey Sher, M.D., Pacific Fertility Medical Center, Los Angeles 10921 Wilshire Boulevard, Suite 700, Los Angeles, CA 90024

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## INTRODUCTION

It has been demonstrated that antiphospholipid antibodies (APA) play a role in reproductive failure including recurrent pregnancy loss (1,2,3), unexplained infertility (1), pregnancy related hypertension (4,5) and intrauterine growth retardation (4). Other studies (5,6,7) link APA to In Vitro Fertilization (IVF) or Embryo Transfer (ET) failure. We previously reported on a negative correlation between APA positivity and IVF outcome and established a therapeutic relevance for the selective administration of mini-dose Heparin/Aspirin therapy (H/A) (8). In a subsequent study we demonstrated that IVIg was beneficial in a subset of women with a specific APA profile undergoing IVF (9). Coulam et. al, reported that the use of IVIg prior to IVF resulted in a 56% success rate among a limited number of patients with multiple failed IVFs (12). The purpose of this study was to help identify criteria for the administration of IVIg in patients who suffered repeated IVF failure. Due to recent controversy regarding the use of H/A in patients undergoing IVF (15), we elected to treat all patients with these drugs elimi-

nating the potential that this variable could impact outcome results when studying the effects of IVIg on these patients.

## MATERIALS AND METHODS

### PATIENT POPULATION

Eighty nine (89) consecutive women who fulfilled the study criteria were included in this study. The inclusion criteria were a) age <36 years, b) four or more failed IVF/ET, c) no male infertility, d) no ovum donation, e) no gestational surrogacy and, f) serum FSH concentration of <15 mIU/ml and a plasma E2 of <70 pg/ml on cycle day three. All patients received gonadotrophin releasing hormone agonist (Lupron, Tapp Pharmaceuticals) for luteal phase pituitary down regulation, followed by menotropin therapy as previously described (10). Starting on day two of controlled ovarian hyperstimulation, each patient received aspirin 81 mg po qd, and heparin 5000 U sq bid. In addition, each patient received 20 gm of intravenous immunoglobulin (IVIg- Gammimune, Bayer Biological or Venoglobulin, Alpha Therapeutic Corp) 3-10 days prior to embryo transfer.

### LABORATORY EVALUATION

All women underwent serum follicle stimulating hormone (FSH) and estradiol (E2) measurements (by radioimmunoassay) on day two or three of a prior menstrual cycle. All women underwent APA testing using an enzyme linked immunosorbent (ELISA) assay for IgM, IgG and IgA isotypes to six phospholipid epitopes (cardiolipin-CL, phosphoserine-PS, phosphoglycerol-PG, phosphoethanolamine-PE, phosphatidic acid-PA, and phosphoinositol-PI) as described previously in detail (11). Borderline positives were defined as >2 SD above the mean of normal controls, and positive values were defined as >3 SD above the mean of normal controls. The control group for the APA assay consisted of non-infertility patients who had no history of clinical or subclinical autoimmune disease, or recurrent pregnancy loss.

Each time the ELISA assay was performed, both known negative and positive controls were run simultaneously for each isotype of every epitope. (This was important to assess the performance of the antigen coated on each plate, the antibody conjugates, the pipetting technique, washing method, incubation times, incubation temperature and substrate).

Cervical or semen specimens were cultured for Ureaplasma, Chlamydia and Gonococcus, in all cases. Male partners all underwent semen analysis and both women and men had sperm antibody serologies measured using the indirect immunobead test.

### DETERMINANTS OF OUTCOME

The number of babies born per transferred embryo was determined in order to provide a measure of the viable im-

plantation rate. Multiple births and miscarriages were documented. A successful IVF outcome was defined as a live birth.

### STATISTICAL METHODS

Data was placed into two-by-two tables: An analysis between and within groups were performed using the Chi Square Test for significance. P values below 0.05 were considered to indicate statistical significance. Analysis was performed using the CHITEST and CHIINV functions for Microsoft Excel 97 for Windows 95.

## RESULTS

Fifty two (52) women were APA+ (Group A), while 37 were APA- (Group B). Table one depicts the homogeneous nature of the patient population studied. Note that there is no difference in the two groups between their age, number of embryos transferred, or miscarriage rate. Also note that there were no differences in the two groups with respect to the etiology of the infertility. Twenty two of fifty two (22/52) (42%) of the Group A patients (APA+) achieved live births following the administration of IVIg, in contrast to only 7/37 (19%) of the women in group B who were APA-. This The results of this study indicate that APA+ women with multiple IVF failures experienced significantly higher birth-rates following the administration of H/A plus IVIg than matched APA- counterparts who underwent the same treat-

**Table 1:** The Effect of Intravenous Immunoglobulin G plus Heparin/Aspirin (H/A) on IVF Birth Rates in 89 Women < 36 years of Age, who underwent 4 Failed IVF Cycles: The Influence of APA Status

	Group A APA +	Group B APA -
Women	52	37
IVF Cycles	52	37
Mean Age	35.1	35.3
Mean # ET per Cycle	5.1	4.8
Baby Rate per Embryo Transferred	11%	5%
Miscarriages	5	5
Multiple Births	8	1
Births	22 (42%)	7 (19%)*
ETIOLOGY		
Unexplained	7	4
Endometriosis	16	12
Pelvic Inflammatory Disease	20	10
Pelvic Adhesions (non-inflam)	9	4

\* Significant Difference: p = 0.020, chi = 5.383

APA = Antiphospholipid Antibodies  
IVIg = Intravenous Immunoglobulin G

ment. We recently reported that APA+ women experience improved IVF implantation and birth rates following H/A therapy provided their APAs were other than IgM or IgG isotypes of anti-PE or anti-PS. In such cases, the addition of IVIg to the regimen significantly improved IVF outcome (9).

Previous reports in the literature suggest that the administration of IVIg benefit women with repeated IVF failures (12, 13, 14). In these studies, the criteria for administering IVIg was based upon the number of prior IVF failures or the total number of embryos that had been transferred during previous IVF attempts without producing a successful pregnancy. As there are side effects associated with IVIg (although not common), in addition to significant costs, we felt it important to establish criteria for the selected use of IVIg in this patient population, so that patients would derive maximum benefit from the therapy.

In developing criteria for selection, the importance of the laboratory technique in performance of the ELISA for the screening test cannot be understated. Although the test can be quite sensitive and specific, if not run properly, it will yield inconsistent and erroneous results. No commercial kit is available that measure the 6 APA epitopes and 3 isotypes tested. Realistically, it takes considerable time to meticulously develop a reliable assay; therefore, these tests should be performed by a laboratory experienced with the APA assay.

It is essential that antibodies to all PL epitopes be determined and measured. Lupus anticoagulant (LA) and APTT are indirect indicators of APA; therefore, are of limited utility. Cardiolipin (CL) was the first PL to be identified and measured directly. Unlike PE and PS, which are surface membrane oriented PL's, CL is expressed primarily on the inner mitochondrial membrane and likely plays a relatively minor role in implantation (trophoblastic syncytialization, etc.). APA levels that are 2 (borderline positive) or 3(positive) SD's above the mean of normal controls respectively have a 95% and 99% probability of being significant. It is critical that specific cut off values be established to ensure optimal specificity without compromising sensitivity.

We do know that phospholipids promote syncytialization of both myoblasts and syncytiotrophoblasts via their adhesion properties (2). Interference with the adhesion properties of phospholipids likely accounts for increase in implantation problems among APA positive patients. The mechanism of action for IVIg in these cases is not clear. Perhaps anti-idiotype antibodies in IVIg might down-regulate segments of the patients immune system and mitigate such things as the toxic effects of cytokines, etc.. The antibodies might also directly interfere with the antiphospholipid antibodies, essentially neutralizing them. It is even possible that APA positivity is an epiphenomena,

and may isolate a group of patient who have polyclonal B cell activation or increase in NK cell activity, which in turn could be amenable to treatment with IVIg.

Whatever the pathophysiology or mechanism of action, the data presented in table I suggest that in women with multiple IVF failures and APA seropositivity, the administration of IVIg resulted in a higher pregnancy rate than women without APA.

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