THE SELECTIVE USE OF HEPARIN/ASPIRIN THERAPY, ALONE OR IN COMBINATION WITH INTRAVENOUS IMMUNOGLOBULIN G IN THE MANAGEMENT OF ANTIPHOSPHOLIPID ANTIBODY POSITIVE WOMEN UNDERGOING *IN VITRO* FERTILIZATION.

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ABSTRACT

PROBLEM: Evaluation of the effect of mini-dose Heparin/Aspirin (H/A) alone vs. combined Intravenous Immunoglobulin G (IVIg) and H/A on In Vitro Fertilization (IVF) birthrates in women who test seropositive for antiphospholipid antibodies (APA+), and whether outcome is influenced by the gammaglobulin isotype(s) or the phospholipid (PL) epitope(s) to which the APA are directed.

METHOD OF STUDY: This was a case-control study conducted in three phases, spanning a four year period. in a multicenter clinical research environment. Six hundred eighty seven APA+ women, < 40 years who each completed up to three consecutive IVF/ET cycles within a twelve month period were given either heparin and aspirin alone, or in combination with IVIg. Birthrates relative to the type of immunotherapy (i.e. H/A alone and H/A+ IVIg) and APA profile were the main outcome measurements.

RESULTS: Phase I: 687 women who tested APA+ to one or more phospholipid (PL) epitopes underwent < 2 IVF attempts for a total of 1050 IVF cycles. 477 births occurred in 923 IVF cycles (46%) where H/A alone was administered. 22 births occurred following 127 IVF (17%) cycles where H/A was not administered. Phase II: 322/687 women tested positive for a single APA sub-type. These subjects underwent up to 2 consecutive IVF attempts for a total of 521 IVF cycles while receiving H/A alone. The birthrate was significantly lower for women whose APA were directed towards phosphoethanolamine (PE) or phosphoserine (PS) involving IgG or IgM isotypes, compared to women who had any other APA (17% vs 43%). Phase III: 121 women who failed to achieve live births following two consecutive IVF attempts where H/A alone was administered, received IVIg in combination with H/A during their third consecutive IVF cycle. The birth rate was 41% following these IVF cycles when anti-PS or anti-PE involving IgG or IgM isotypes were present, as compared with 17% when H/A alone was administered. IVF outcome didn't improve when IVIg was administered in association with any other single APA.

CONCLUSION: Treatment of APA+ women with H/A alone improves IVF birthrates. This benefit is selective in that it does not apply in cases where IgG or IgM related APA are directed against PE or PS. In such cases, the addition of IVIg significantly improves outcome.

Key Words: Birthrate, In Vitro Fertilization, Antiphospholipid Antibodies, Heparin/aspirin and Immunoglobulin G.

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AJRI 1998: 40:74-82

INTRODUCTION

Despite the introduction of enhanced protocols for ovarian stimulation, improved embryo culturing techniques, assisted hatching and intracytoplasmic sperm injection, the national average birthrate per completed cycle of In Vitro Fertilization (IVF) and Embryo Transfer (ET) has minimally improved over the last few years and is still not much above 20% per egg retrieval (1). The successful implantation of a morphologically normal embryo transferred to the uterus remains the bottleneck in every IVF program.

For several years, researchers have shown a link between abnormalities relative to the woman's immune system and recurrent pregnancy loss (2-5). More recent data suggests that the immunologic pathophysiology associated with recurrent pregnancy loss and early implantation failure may be similar. Clearly, implantation failure at a preclinical stage is often misdiagnosed as "infertility". Perhaps the term Failed Pregnancy Recognition (FPR) would be more appropriate in such cases. It is likely that immunologic deficiency plays a significant role in a variety of implantation related problems, including but not limited to; FPR, biochemical pregnancy, blighted ovum and first trimester miscarriage (4-9).

Bustillo, et al. reported a five fold increase in the prevalence of APA seropositivity in 84 women who failed to conceive following the transfer of twelve or more embryos over a number of IVF/ET cycles (10). Sher, et al. described a high prevalence of antiphospholipid antibodies (APA) in women with pelvic organic disease who underwent in vitro fertilization (IVF) and embryo transfer (ET), and demonstrated a three fold improvement in clinical PR with the first IVF cycle when H/A was administered to APA+ women under the age of 40
years (7), suggesting that autoimmune mechanisms associated with early implantation failure are potentially treatable.

It has been demonstrated that APA+ patients undergoing IVF benefit from H/A therapy (7), therefore, we tested all IVF candidates prior to commencing their first IVF attempt. Over the years, we noted a trend from the results in our clinic suggesting that women who possessed IgG or IgM antibodies to phosphoserine (PS) or phosphoethanolamine (PE), had lower pregnancy rates than women with any other APA. With this observation, and Coulam, et. al. reported success using intravenous immune globulin (IVIg) on unselected patients who had failed several IVF attempts (11), we attempted to establish specific criteria for which patients might benefit from H/A along with IVIg. IVIg was felt to be an appropriate immune modulator because it has been proven useful in a variety of autoimmune disorders such as Kawasaki's disease, idiopathic thrombocytopenic purpura and Wiskott-Aldrich syndrome. Although the mechanism of action is unclear, it is believed that the anti-idiotype antibodies in the IVIg play an immune modulating function (12). The specific objectives of this analysis are: 1) To determine whether the effect of H/A therapy was influenced by the phospholipid (PL) epitope against which the APA were directed as well as the gammaglobulin isotype involved; and 2) To ascertain whether H/A treated, APA+ women who failed to conceive following two consecutive IVF cycles of treatment, would experience improved birthrates following the combined administration of H/A and IVIg during the third consecutive IVF attempt, and to establish if any particular APA type predicts which women may benefit from IVIg therapy.

MATERIALS AND METHODS

A study was undertaken, involving 687 APA+ women (age range 29-40 years, mean age 36.25 years) who underwent IVF/ET at Pacific Fertility Medical Center during a four year period commencing January 1992. Cases of male infertility, ovum donation and gestational surrogacy were excluded. The data analysis was conducted in three phases.

Phase I
Six hundred eighty seven (687) women who tested APA+ to one or more PL epitope each underwent < 2 IVF cycles for a total of 1050 completed treatment cycles. All women were offered treatment with heparin and aspirin. Those who accepted were put into Group A and those that declined were placed in Group B. Six hundred three (603) of these women (Group A) underwent a total of 923 IVF cycles of treatment where H/A alone was administered, while the remaining 84 women (Group B) underwent 127 IVF cycles where H/A was not administered.

Phase II
We evaluated whether IVF outcome following two consecutive cycles of H/A therapy was influenced by the APA profile (i.e. the specific PL epitope to which APA were directed and the associated gammaglobulin isotype). Since the number of individual APA sub-profiles was quite large, the sub-groups were too small to permit statistical comparison in patients who had APA directed against multiple phospholipids. In addition, it would be difficult to ascertain if one specific PL influenced outcome in the presence of other PLs. Accordingly, Group C represents those 322 women from Group A who each tested positive to only one APA.

Phase III
Group D represents one hundred twenty one (121) women from Group C who did not achieve viable pregnancies in Phase II (i.e. following two consecutive IVF attempts), where immunotherapy was confined to H/A alone. These

women received IVIg in combination with H/A during their third consecutive IVF cycle.

LABORATORY EVALUATION
All women underwent serum follicle stimulating hormone (FSH) and estradiol (E2) measurements (by radioimmunoassay or enzyme assay) on day two or three of a prior menstrual cycle. Only those women who had a serum FSH concentration of < 10mIU/ml and a plasma E2 of < 70pg/ml on cycle day three, were included in the study. All women underwent APA testing using an enzyme-linked immunosorbent assay for antibodies to the following six phospholipid epitopes: cardiolipin (CL), phosphoserine (PS), phosphoglycerol (PG), phosphoethanolamine (PE), phosphatidic acid (PA), and phosphoinositol (PI), as previously described in detail (13). Antiphospholipid antibody seropositivity (APA+) was defined by the detection of APA measuring > two (2) standard deviations from the mean to IgA, IgM and/or IgG isotypes. Borderline values were defined as >2 SD above the mean, 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 epitope and isotype. 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 analyses to exclude male factor infertility.

DETERMINANTS OF OUTCOME
The number of babies born per transferred embryo, was determined in order to provide a measure of the viable implantation rate. Multiple births and miscarriages were documented. A successful IVF outcome was defined as a live birth.

TREATMENT
All patients underwent controlled ovarian hyperstimulation (COH) with menotropins, preceded by pituitary down regulation with gonadotrophin releasing hormone agonist (GnRHa) by a previously described protocol (14).

All patients included in this study were APA+ and received aspirin 81 mg po qd and heparin 5000 U sq bid, starting on day two of Controlled Ovarian Hyperstimulation. Heparin was withheld on the morning of egg retrieval, and reinstated that same evening while aspirin was withheld from the ninth day of menotropin stimulation and was reinstated on the evening following egg retrieval. H/A was discontinued if pregnancy did not occur as evidenced by rising quantitative human chorionic gonatotropin (HCG) blood levels measured respectively on the 8th and 10th day post-ET. Patients with a positive HCG blood test continued with H/A treatment through the diagnosis of a clinical pregnancy, 3-4 weeks post ET. H/A was continued until, at least the 10th gestational week. H/A treatment was monitored as previously described (7).

STATISTICAL METHODS
Data was placed into two-by-two tables; and analyses between and within groups were performed using the Chi Squared test for significance. P values below 0.05 were considered to indicate statistical significance. Analysis was performed using the CHITEST and CHIINV functions of Microsoft Excel 97 for Windows.

RESULTS

Phase I
Table 1 illustrates that patients of Groups A&B were comparable with regard to; mean age, mean number of embryos transferred, and mean number of IVF failures prior to initiating treatment. In Group A, 417/923 or 46% of the IVF cycles, resulted in live births as compared to a birthrate of 22/127 (17%) in Group B. The difference is highly significant ($P<0.0001$) and points to a clear benefit associated with H/A therapy in the APA+ women who underwent IVF. The mean multiple birthrate was 32% for Group A vs. 18% for
Group B, suggesting that H/A therapy improved the implantation rate in APA+ patients. The viable birth rates per embryo transferred were 15% for Group A and 4% for Group B respectively while the miscarriage rate was 14% for Group A and 18% for Group B.

Phase II
Table 2 presents the APA profiles of 322 women (Group C), who each tested positive for antibodies to a single PL epitope. The number of patients in each subgroup was too small to allow meaningful statistical analysis. Accordingly, in order to test our hypothesis that the IgG and IgM isotypes of anti-PS or anti-PE antibodies are associated with poor outcomes, we tested all possible combinations of subtype pairs in each isotype category to see if there was a difference in success rates, using chi-square analysis. As seen in Table 3, only when antibodies directed towards PE and PS were combined in the IgG and IgM groups, did we see statistically lower birth rates. The mean number of prior failed IVF cycles, the mean number of embryos transferred per ET, the mean ages of patients and the etiology of infertility were similar in each group, although a slightly higher number of embryos were transferred in the anti-PE/PS IgM group. Despite this, the birth rate was still lower in this group. Table 3 illustrates that women with IgG or IgM related APA, directed against PE or PS experienced an overall birthrate of 17% (21/124) per cycle and a live birth rate

Table 1: Influence of Heparin/Aspirin Therapy on IVF Outcome in 687 APA+ Women who completed 1050 IVF/ET Cycles

	Group A with H/A Therapy	Group B without H/A Therapy	PValue (c2)
Number of women	603	84	
Number of IVF/ET cycles	923	127	
Mean number of prior IVF failures	1.7	2.2	
Mean age	35.7	36.8	
Baby rate per embryo (%)	15	5	
Mean no. of embryos transferred per cycle	4	4.6	
Number of miscarriages	68	5	P = 0.154 (2.031)
Number of multiple births	133	4	P < .001 (12.475)
Number of births per embryo transfer (%)	417 (46%)	22 (17%)	P < .001 (35.606)
ETIOLOGY of INFERTILITY			
Unexplained	417 (45%)	22 (17%)	
Endometriosis	303 (32%)	42 (33%)	
Pelvic inflammatory disease	313 (34%)	38 (30%)	
Pelvic adhesions	141 (15%)	28 (22%)	

per embryo transferred of 5%. In contrast, women whose APA were of any other profile, had a birthrate of 47% (170/397) and a mean live birth rate per embryo transferred of 12%. These findings suggest that the APA profile influenced both implantation rates and birthrates in this patient population group.

Phase III

Table 4 presents the demographic characteristics of 121 women (Group D) whom, following two consecutive IVF failures where H/A (alone) had been administered, completed a third cycle of treatment where H/A plus IVIg was given. The demographic characteristics were relatively homogenous. Table 4 illustrates that patients who had IgG or IgM related APA directed specifically towards PE or PS had a significant increase in IVF birthrate following the addition of IVIg to H/A therapy (i.e. from 21/124 cycles [17%] in Phase II, to 18/44 cycles [41%]) in Phase III, P < 0.0001, while women whose APA were directed towards any other PL epitope or were IgA related, did not experience an improved IVF birthrate (170/397 cycles [43%] vs 28/77 [36%]), P>0.05.

Table 2: Influence of APA subtype and gammaglobulin isotype on IVF outcome in 322 women < 40 years of age, who tested positive for a single APA

	Anti-PE	Anti-PS	Anti-PG	Anti-PA	Anti-PI	Anti-CL	Total
IgA	16	24	19	20	17	11	107
No. of pregnancies	7	10	7	9	6	5	44
PR (%)	43.8	41.7	36.8	45.0	35.3	45.3	41.1
IgM	18	14	17	18	12	19	98
No. of pregnancies	3	3	8	9	7	9	39
PR (%)	16.7	21.4	47.1	50.0	58.3	47.3	39.8
IgG	17	16	19	22	19	24	117
No. of pregnancies	4	4	10	11	9	10	48
PR (%)	23.5	25	52.6	50.0	52.6	50.0	41.0

Note:, IgA=Immunologlobulin A, IgG=Immunoglobulin G, IgM=Immunoglobulin M, PA=Phosphatydic Acid, PE=Phosphoethanolamine, PG=Phosphoglyceryol, PI=Phosphoinositol, , PS=Phosphoserine, CL=Cardiolipin, PR=Pregnancy Rate

Table 3: Influence of APA Profile and Gammaglobulin Isotype on IVF Outcome, following H/A Therapy (322 women who each underwent <2 IVF Cycles)

	IgA		IgM		IgG		
	Antibody to PE/PS+	Antibody to PE/PS-	Antibody to PE/PS+	Antibody to PE/PS-	Antibody to PE/PS+	Antibody to PE/PS-	P Value (c2)
No. of women	42	61	38	59	35	87	
No. of IVF/ET cycles (n=521)	63	108	67	104	57	122	
Mean no. of prior IVF failures	1.2	1.1	3.8	1.5	2.6	2.9	
Mean age	36.1	34.9	37.4	35.9	38.5	35.1	
Baby rate per embryo (%)	11	15	4	8	5	10	P = 0.219,chi = 1.513
Mean no. of embryos per embryo transfer	5.5	4	4	4.9	4.2	5.3	
No. of miscarriages	4	7	4	9	3	7	P = 0.207,chi = 0.207
No. of multiple births	12	22	1	15	1	18	P < .001,chi = 19.159
No. of births (%)	26 (41)	42 (39)	10 (15)	47 (45)	11 (19)	45 (37)	P < .001,chi = 11.475
ETIOLOGY of INFERTILITY							
Unexplained	16%	15%	12%	22%	18%	10%	
Endometriosis	19%	35%	15%	25%	45%	32%	
Pelvic inflammatory disease	19%	23%	22%	31%	9%	44%	
Pelvic Adhesions	22%	11%	10%	23%	28%	22%	

Note: The influence of antibodies to PE/PS+ involving IgG and IgM profiles was compared to the influence of all other antibodies.
Note: IgA=Immunologlobulin A, IgG=Immunoglobulin G, IgM=Immunoglobulin M, PE=Phosphoethanolamine, PS=Phosphoserine

Table 4: Influence of APA Profile and Gammaglobulin Isotype on Outcome, following H/A (Heparin/Aspirin) + IVIg in 121 Women during their 3rd consecutive IVF/ET Cycle

	IgA		IgM		IgG		
	Antibody to PE/PS+	Antibody to PE/PS-	Antibody to PE/PS+	Antibody to PE/PS-	Antibody to PE/PS+	Antibody to PE/PS-	P Value (c2)
No. of women	9	22	25	25	19	21	
No. of IVF/ET cycles	9	22	25	25	19	21	
Mean no. of prior IVF failures	3.0	2.9	4.5	3.1	4.5	4.1	
Mean age	36.0	34.8	37.5	36.5	36.5	35.4	
Implantation rate per embryo	10.0	12.0	8.0	9.0	14.0	8.0	P = 0.945,chi = 0.005
Mean no. of embryos per embryo transfer	5.7	4.2	4.8	5.4	4.1	5.3	
No. of miscarriages	0	1	0	2	1	1	P = 0.437,chi = 0.604
No. of multiple births	1	2	1	3	2	3	P = 0.389,chi = 0.743
No. of pregnancies (%)	4 (44)	9 (41)	9 (36)	9 (36)	9 (47)	6 (29)	P = 0.629,chi = 0.246
ETIOLOGY of INFERTILITY							
Unexplained	--	18%	20%	20%	16%	--	
Endometriosis	--	44%	24%	24%	37%	33%	
Pelvic inflammatory disease	56%	--	24%	24%	26%	19%	
Pelvic Adhesions	44%	24%	32%	32%	21%	48%	

Note: The influence of antibodies to PE/PS+ involving IgG and IgM profiles was compared to the influence of all other antibodies.
Note: IgA=Immunologlobulin A, IgG=Immunoglobulin G, IgM=Immunoglobulin M, PE=Phosphoethanolamine, PS=Phosphoserine

The data suggests that the observed improvement in the viable implantation rate (baby rate per transferred embryo) and IVF outcome associated with the addition of IVIg to H/A therapy, occurred selectively in women who had IgG or IgM related APA directed against PE or PS, and were therefore, "H/A poor responders".

DISCUSSION

We previously reported on the observation that 53% of women <40 years of age, with organic pelvic pathology as an indication for IVF, tested APA +, as compared with 14% in a control group comprised of women who underwent IVF exclusively for the treatment of male factor infertility. Forty eight percent of the APA + women achieved clinical pregnancies following a single IVF cycle where H/A was administered (7). In 1996, Kaider et al. reported on the high prevalence of APA positivity in women who had experienced repeated IVF failures (15). The present study is strongly suggestive of an overall benefit associated with H/A immunotherapy in the treatment of APA+ women undergoing IVF (Table I, Figure I). The current study further demonstrates that women who have IgG or IgM related APA that are specifically directed toward PE or PS had improved birthrates and increased implantation rates following the addition of IVIg to the H/A regimen, while no such effect was apparent when IVIg was administered in association with any other APA profile. Interestingly, when anti-PE or anti-PS antibodies involved the IgA isotype, the addition of IVIg afforded no apparent therapeutic benefit. Since IgA fractions are found in most human secretions, it is possible that heparin more readily gains access to intracellular PL target sights in order to repel IgA, APA.

We cannot say, at this time, whether the presence of APA is causally linked to implantation failure, or whether their existence represents a theoretical marker for some other pathological process. It is our contention that the presence of APA adversely affects IVF birth rates when the cause of the infertility is due to a presumed female factor. We are reluctant to suggest that the same may be true for couples with isolated male infertility.

The results of this study would suggest that patients who have APA involving IgM and IgG isotypes can, on the basis of IVF outcome, be divided into two distinct subgroups. One group clearly comprises potential H/A responders, while the other, are H/A non-responders.

It is highly likely that immunotherapy administered to APA+, IVF patients affect events that influence implantation of the blastocyst. The fact that some patients respond to H/A alone while others require the addition of IVIg strongly suggests that the mechanisms associated with implantation failure caused by APA positivity are multiple.

Partially based upon previous research involving recurrent miscarriages, it is postulated that the mechanisms involved in APA induced IVF failure, may be due to an interference with the adhesion properties of phospholipids involved in trophoblastic syncytialization and/or to endovascular thrombosis within the microvasculature supplying the choriodecidual space. (7,16). This hypothesis is strengthened by the fact that another phospholipid, Platelet Activation Factor (PAF) has been shown to enhance clinical PR's when cultured with human IVF embryos. (17).

The results of this study demonstrate that the H/A non-responders had such a comparatively poor clinical PR in the second IVF cycle that clearly another mechanism for IVF failure must be involved. We observed that the addition of IVIg to the treatment regimen in the third IVF/ET cycle, resulted in a significant improvement in clinical PR's.

Several studies have suggested that the use of IVIg improves clinical PR's in patients with recurrent spontaneous abortions (RSA), (19-21). Three groups have reported on placebo controlled trials (one randomized) where IVIg was administered to women who had repeated IVF/ET failures. De Placido, et al. reported a three fold improvement in embryo implantation rates (17.7% vs. 8.0%) following administration of IVIg (21). Kleinstein, et al. demonstrated a six fold enhancement in clinical PR's (50% vs. 8%) when immunoglobulin G was given (22). Coulam, et al. reported a significantly improved outcome with IVF when IVIg therapy was employed in association with repeated IVF failure (11). Based upon these findings, IVIg has been used empirically, with some success, to treat women who have had repeated IVF failures.

It is now known that large numbers of Natural Killer (NK) cells are present in the placenta, and in close proximity to the extravillous trophoblast at the site of implantation. Normally, these cells are not cytotoxic, and secrete various cytokines, including GM-CSF and TNF alpha and beta, which appear to be important to the maintenance and growth of the trophoblast (23). Recently it has been reported that TNF alpha plays a key role in trophoblast migration (24). Of note, women with markedly elevated levels of Natural Killer (NK) cells have an increased incidence of Recurrent Spontaneous Abortions (RSA) and it has been shown that IVIg decreases the activity of NK cells in such cases, which may be a reason why IVIg has been successful in the treatment of patients with RSA (21). If we carry this same hypothesis further, then IVIg, by decreasing NK cell activity, might likewise enhance implantation in patients with repeat IVF failure due to FPR.

Based upon the data presented regarding APA profiles in patients undergoing IVF, we conclude that women who had IgM and IgG APA to PE or PS responded poorly to H/A alone, but did well when IVIg was added to the treatment regimen. It has been known for years that the surface phospholipids, PE and PS, principally through their adhesion

properties, promote syncytialization of both myoblasts and syncytiotrophoblasts (16, 25, 26). It is possible that in this situation the presence of APA to PE or PS may be an epiphenomenon, acting as a marker to one or more other significant autoimmune processes (such as an increase in NK cell activity) which in turn might interfere with early implantation. In addition, we suggest that that immunoglobulin G, through its inherent anti-idiotype antibodies, might modulate the autoimmune process by suppressing CD19 B cell activation and/or by directly blocking autoantibodies.

The importance of the laboratory technique in performance of the ELISA assay cannot be understated. Although the test can be quite sensitive and specific, if not run properly, it will yield inconsistent and erroneous results. This is true from the very start of the procedure, including: a) choice of plastic ELISA plates (various plastics have different affinities for proteins), b) the amount of antigen plated, c) the choice and dilution of a blocking buffer that blocks non-specific sites on the plate without increasing the background, d) the dilution of antibody conjugate that bind to antigen without binding to non-specific sites, e) the washing technique (automated plate washer recommended to assure consistency), f) incubation with substrate so that the color reaction allows differentiation between positive and negative values, and g) the interpretation of raw data which presumes that appropriate control and study groups are selected, and that background noise is subtracted out, etc. No commercial kit is currently available that measures the 6 APA epitopes and 3 isotypes tested. It takes considerable time to meticulously develop a reliable assay. It is tempting to transgress from the usual methodology, resulting in erroneous, unreproducible and possibly misleading data, which was the case with the recently reported study by Denis, et al on APA profiles in IVF patients (27). Denis, used unusually high concentrations of antigens to coat the trays; did not compensate for different plastic-antigen affinities; and stopped the reactions prematurely. There were other serious design problems as well.

It is essential that antibodies to all PL epitopes be determined and measured. Studies that focus upon a single APA type, while ignoring the others, should not be regarded as valid, since they discount the importance of the other antibodies, which may be more significant than expected. Lupus anticoagulant and APTT are indirect indicators of APA and therefore are of limited utility. Cardiolipin was the first PL to be identified and measured directly. Unlike PE and PS, which are surface membrane oriented PL's, Cardiolipin 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) 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 comprising sensitivity.

This is not the first study to demonstrate that the use of IVIg could improve implantation and clinical PR in patients undergoing IVF (11,22-23). However, this is the first study to define preliminary indications for such treatment.

CONCLUSION

In summary, we conclude that: H/A therapy alone is adequate for the treatment of APA seropositive women who fit the following criteria:

1. APA are of the IgA isotype, regardless of the PL epitope to which they are directed.
2. APA positivity of the IgM or IgG isotypes is directed towards PL's other than PE and PS. The addition of IVIg to the therapeutic regimen is recommended when APA of the IgM or IgG isotypes are directed at PE or PS.

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