THE USE OF COMBINED HEPARIN/ASPIRIN AND IMMUNOGLOBULIN G. THERAPY IN THE TREATMENT OF IVF PATIENTS WITH ANTITHYROID ANTIBODIES.

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ABSTRACT
PROBLEMS: To compare the effect of Heparin Aspirin (H/A) therapy alone vs. H/A in combination with Intravenous Gammaglobulin (IVIg) immunotherapy on IVF outcome in patients who test positive for antithyroid antibodies (ATA).
METHODS: We evaluated 82 women less than 40 years of age whose infertility was exclusively related to female causes. All tested positive for organ-specific antithyroid antibodies (antimicrosomal and/or antithyroglobulin antibodies), but negative for antiphospholipid antibodies. Thirty-seven (37) of these women (Group A) received H/A alone, while 45 (Group B) received H/A in combination with IVIg.
RESULTS: Ten (10) or 27% of Group A and 23 (51%) of Group B women, achieved live births following completion of a single IVF/ET cycle (P=0.027).
CONCLUSIONS: We conclude that IVIg therapy significantly improves IVF success rates in ATA+ women.

Key Words:
In Vitro Fertilization and embryo transfer (IVF/ET), Immunoglobulin G (IVIg), antithyroid antibodies (ATA)

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AJRI 1998: 39:223-225

INTRODUCTION

A relationship between antithyroid antibodies (ATA) and reproductive failure has been established. In 1990, Stagnaro-Green evaluated a selected obstetric population with a prior history of poor reproductive performance, and was able to show a relationship between antithyroid antibodies and miscarriage. (1). This was subsequently confirmed by Glinoer, et al. in 1991 (2). It was later demonstrated that women who have an increased concentration of antithyroid antibodies and recurrent pregnancy loss do not necessarily demonstrate anticardiolipin antibody (3). Recently, Geva, et al. demonstrated that more than 20% of 78 patients undergoing IVF for mechanical or unexplained infertility tested positive for antithyroid antibodies, and 12% were positive for antiovarian antibodies. Of note, is the fact that all patients in that study were clinically euthyroid with no history of having been medicated for hypothyroidism (4). This data suggest that antithyroid antibodies may be independent markers for reproductive failure.

It has been suggested that the existence of antithyroid antibodies, before or during early pregnancy may reflect activated T cell function, which in turn may be related to TH1 lymphocytes (3,5).

In designing this study, we wished to examine the efficacy of only one variable

(the use of IVIg) on outcome in IVF patients who demonstrated thyroid antibodies. Because of the recent controversy over the use of aspirin and heparin in patients undergoing IVF (8, 9), we elected to treat all patients with aspirin and heparin, thereby eliminating the potential that this variable could have an impact on outcome results when studying the effects of IVIg on these patients.

MATERIALS AND METHODS

PATIENTS

A prospective study was undertaken to evaluate whether reatment with Heparin/Aspirin alone versus combined H/A + IVIg would influence IVF success rates.

Eighty two (82) women < 40 years of age, who tested positive for ATA, but negative for antiphospholipid antibodies (APA) were randomly placed into two groups in a non-discriminating quasi alternating fashion. Cases of male infertility, ovum donation, and gestational surrogacy were excluded. Group A comprised 37 women who received H/A alone while Group B consisted of 45 women who received H/A in combination with intravenous immunoglobulin G (IVIg – Gammimune, Bayer Biological or Venoglobulin, Alpha Therapeutic Corp) 7-14 days prior to embryo transfer.

Patients who had abnormally low plasma levels of IgA were considered to be at risk for the development of anaphylaxis and were selectively medicated with antihistamines and corticosteroids prior to and during the 2-3 hour IVIg infusion. A second infusion of IVIg was given upon the chemical diagnosis of pregnancy through quantitative serum HCG measurement and a final IVIg infusion was performed upon ultrasound confirmation of a viable pregnancy (between the 6th and 7th gestational week). All patients underwent controlled ovarian hyperstimulation (COH) using premenstrually administered gonadotropin releasing hormone agonist (lupron-Tapp pharmaceuticals), followed by menotropin therapy, as previously described (7). The measurement of APA's was performed as previously described by Matzner, et al. (8).

Antithyroid antibody positivity (ATA+) was defined by the detection of antithyroglobulin and/or antimicrosomal antibodies as measured by the QUANTA Lite Thyroid T and Thyroid M ELISA assay from INOVA Diagnostics (San Diego, CA). Briefly, 100 microliters of prediluted controls or diluted samples were added to the microwell plates (which were coated with thyroglobulin or microsomal antigen at the factory), and incubated at room temperature for 30 minutes. The plates were washed in a wash buffer three times, and 100 microliters of HRP Conjugate was added to each well. The plates were then incubated for another 30 minutes. The plates were again washed three time,s and 100 microliters of TMB Chromogen was added to the wells, and incubated for 30 minutes. At that time, 100 microliters of stopping solution was added, and the absorbance read at 450 nm, using 550 nm as a reference wavelength. The published relative sensitivity for this assay is 96.8%, and the relative specificity is 94.7%.

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.

STATISTICAL METHODS

Data was placed into two – by – two Tables: And analysis between and within groups was 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 for Microsoft Excel 97 for Windows.

RESULTS

Table I compares Groups A&B with regard to demographic characteristics and IVF outcome. The IVF birthrate per embryo transferred was significantly greater for Group B than Group A [23/45 (51%) vs. 10/37 (27%)] p=0.027. There

Table I: A Comparison of the Influence of Heparin/Aspirin (H/A) Alone versus Combined H/A and Intravenous Immunoglobulin G (IVIg) Therapy on IVF Outcome in 82 Women Who Tested Negative for Antiphospholipid Antibodies (APA-) and Positive for Organ Specific Antithyroid Antibodies (ATA+)

	Group A H/A alone	Group B HA + IVIg
Women	37	45
IVF Cycles	37	45
Mean Age (yrs.)	35.7	34.9
Mean # ET per Cycle	3.9	4.5
Live Birth Rate per embryo transferred	8%	17%
Miscarriages	4	4
Multiple Births	2	6
Births	10(27%)	23(51%)*
ETIOLOGY		
Unexplained	32%	27%
Endometriosis	22%	31%
Pelvic Inflammatory Disease	22%	36%
Pelvic Adhesions (non-inflam)	24%	7%
Clinical Hypothyroidism	6(17%)	9(20%)

* Significant Difference: p = 0.027, chi = 4.8975

APA = Antiphospholipid Antibodies
IVIg = Intravenous Immunoglobulin G
ATA = Antithyroid Antibodies

were no significant differences in the other demographic characteristics noted in Table I. It is notable that 6 (17%) of the 37 women in Group A and 9 (20%) in Group B had clinical evidence of hypothyroidism.

DISCUSSION

It has long been recognized that women who test positive for organ specific anti-thyroid antibodies have a high incidence of reproductive failure, as evidenced by recurrent miscarriages and a relatively low pregnancy rate following advanced fertility treatment (1-4). This also explains the high IVF failure rate in patients who test positive for organ specific antithyroid antibodies. In fact, it may be appropriate to consider such IVF failures as being due to Failed Pregnancy Recognition (FPR) rather than to poor egg or embryo quality. The distinction between IVF failures due to FPR and those attributable to embryo or gamete insufficiency is important because failure to implant carries with it the implication that subsequent placental reserve may be compromised, thereby impacting fetal well being and potentially the quality of life after birth.

It is possible that ATA's, like APA's directly compromise trophoblastic development. However, it has been suggested that the relationship may be indirect in that the presence of such antibodies may simply represent a marker for increased T-Cell activation and toxic cytokine production by TH1 lymphocytes. (5).

The anti-idiotype antibodies contained in IVIg, by neutralizing ATA's, might mitigate the toxic effects of cytokines. Likewise, heparin and aspirin, through antithrombotic and anticoagulant properties, might prevent vascular thrombosis in the choriodecidual vasculature, and promote healthy implantation.

Whatever the pathophysiology or mechanism by which these immunotherapies operate, it is clear from the results in this study, that IVF patients who test positive for organ specific antithyroid antibodies experience significantly improved IVF outcomes, when H/A and IVIg are administered prior to egg retrieval.

We cannot say that ATA+ women would or would not benefit from aspirin/heparin alone. Further studies are needed to look at outcome using IVIg without aspirin/heparin. In this study, aspirin/heparin was deliberately administered to all patients to mitigate the effect that that therapy might have on outcome while testing the effectiveness of IVIg in ATA+ patients., Nevertheless, based upon the data presented in Table I we conclude that:

IVIg + H/A-treated ATA+ patients (Group B) had a significantly higher IVF birthrate as compared to those ATA+ women who received H/A alone (51% vs. 27%). P=0.027.

Treatment of antithyroid antibody-positive patients with IVIg significantly improved IVF outcome.

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