Article

Treatment of recurrent IVF failure and human leukocyte antigen similarity by intravenous immunoglobulin

Tamar Elram received her MD in 1999 from The Hebrew University in Jerusalem. She is currently a resident in obstetrics and gynaecology at Hadassah Medical Center, Israel. Her research is in the fields of infertility and obstetrics, with emphasis on couples with prolonged infertility treatment failure; she is investigating immunological methods for improving success rates. In obstetrics, she is part of a team that has established a multidisciplinary approach designed to decrease morbidity and mortality from severe cases of placenta accreta. Current studies involve attempts to reduce costs and hospitalization rates for women at risk for maternal postpartum infections. Other interests include external cephalic versions and the role of oxidative stress in diabetic pregnancy.

Dr Tamar Elram

T Elram1,3, A Simon1, S Israel2, A Revel, D Shveiky, N Laufer
1Department of Obstetrics and Gynecology; 2Department of Tissue Typing, Hadassah University Hospital, Ein Kerem, Jerusalem, Israel
3Correspondence: Tel: +972 2 6777111; Fax: +972 2 6433337; e-mail: coren-elram@barak-online.net

Abstract

This study sought to assess the efficacy of intravenous immunoglobulin (IVIg) in improving pregnancy rates and outcome, in a select group of patients with repeated IVF failure and human leukocyte antigen (HLA) similarity. Couples suffering from recurrent IVF failure, defined as at least seven attempts at embryo transfer with no successful implantations, who were found to share at least three HLA loci, and a negative cross-match test, were included in the study. The treatment consisted of two 30 g IVIg doses: one before oocyte retrieval, and a second as soon as a fetal pulse was identified on ultrasound. Ten couples comprised the study group. In total, these couples had undergone 98 IVF cycles with no successful pregnancies prior to initiation of the study. Following a total of 18 IVIg courses, seven women conceived, two women twice. Up to date, five women have delivered at least one live fetus, at 27 weeks or later. One woman is currently in the early third trimester of a twin pregnancy, and one woman had a late abortion at 19 weeks. The results suggest that couples with recurrent IVF failure and HLA similarity, may benefit from IVIg treatment.

Keywords: HLA loci, intravenous immunoglobulin, recurrent IVF failure

Introduction

The maternal immune system plays a major role in the establishment and maintenance of a normal pregnancy (Daunter, 1992; Weetman, 1999; Szekers-Bartho, 2002). Both local and systemic immunological factors have been identified that decrease the immunogenicity of the allogenic blastocyst and/or alter the maternal immune response to facilitate implantation and the maintenance of early pregnancy (Wegmann et al., 1993; Rouas-Freiss et al., 1997; Stephenson and Fluker, 2000). Fetal antigens that are expressed at the maternal–fetal interface, elicit a maternal immune response mechanism which is vital in protecting the fetus from immune rejection. Blocking antibodies and the resultant maternal–fetal tolerance appear in successful pregnancies, while abortions may occur in the absence of these factors (Fraser et al., 1993). A manifestation of this immune response is the maternal ability to express anti-paternal antibodies. The presence of these antibodies is determined by crossmatching between maternal undiluted fresh serum with paternal lymphocytes (Mittal et al., 1968). A positive maternal serum kills freshly drawn paternal peripheral lymphocytes at a proportion >40% of the control negative serum (Orgad et al., 1999). Crossmatch negative couples with either recurrent spontaneous abortions or IVF failure may be candidates for paternal leukocyte immunization as a means of stimulating the maternal immune response towards the fetus (Fraser et al., 1993). Excessive antigen sharing contraindicates the use of paternal leukocyte immunization, since this may prevent the recognition of foreign antigens and hence prevent seroconversion to anti-paternal antigen positive, and may lead to graft versus host disease (Carp et al., 2001). A high rate of human leukocyte antigen (HLA) loci sharing is an expression of genetic similarity and may act to prevent the appearance of
these essential antibodies. The relevant HLA loci (A, B, C, DR, DQ) are those presenting peptides that come in contact with the partner’s T cells and initiate the immune response cascade (Li et al., 2004). Most couples will share no more than one loci (of 10 alleles inherited from both parents). Several reports have suggested that genetic similarity, expressed as increased HLA loci sharing, may lead to adverse pregnancy outcome including recurrent miscarriages, low birth weight and pre-eclampsia (Weckstein et al., 1991; Balasch et al., 1993; Ho et al., 1994; Sbracia et al., 1996). A number of studies have also demonstrated a significant excess of HLA sharing among couples failing multiple inducton of ovulation and intrauterine insemination cycles compared with those who conceive (Weckstein et al., 1991; Balasch et al., 1993; Ho et al., 1994). Expression of the HLA class I gene HLA-G has been shown to affect pregnancy outcome in several ways, though this field of research involves only the maternal or embryonal immune system and does not look into the interaction with the paternal HLA system (Warner et al., 2002; Sher et al., 2004).

Intravenous immunoglobulin (IVIg) is a monomeric IgG preparation, produced from the plasma of thousands of blood donors that has been used for a variety of immunological disorders since 1980 (Beer and Kwak-Kim, 2001). Being a pooled preparation, it has a diverse antibody profile. Several mechanisms have been suggested considering the mode of action of IVIg in modulating the immune system. Some of these proposed mechanisms could theoretically improve implantation and the maintenance of an early pregnancy through enhanced production of T-helper 2 cytokine production, inhibition of natural killer cell activity and increased antibody production (Loke and King, 1995; Creus et al., 1998). Summary of the data in an ASRM Practice Committee Report, from 2001, found the effectiveness of IVIg as a treatment for recurrent spontaneous abortion (RSA) to be unproven; nevertheless, a potential effect was demonstrated among couples with secondary RSA.

A number of studies report experience with this mode of treatment in cases of recurrent IVF failure. The results suggest that IVIg may be useful in the treatment of unexplained IVF failure in women with good previous fertilization rates and embryo production (Coulam et al., 1994; De Placido et al., 1994), though a randomized placebo controlled trial (Stephenson and Fluker, 2000) found no improvement in the live birth rate following IVIg treatment. Although these studies do not recommend the routine use of IVIg for repeat IVF failure, none has focused on HLA similarity as a subgroup that deserves special attention.

Two reports include HLA testing for infertility treated couples (Balasch et al., 1996; Scher and Salazar, 2000). The former study reported experience with IVIg treatment for recurrent IVF failure in 12 patients, noting that two women shared three or more HLA loci with their spouse. No implantations occurred, and they concluded that high dose IVIg is not useful in IVF–embryo transfer failure. The latter study reported experience with patients treated with IVIg following recurrent IVF failure, significantly improving pregnancy rates among groups with different indications for IVF, including recurrent spontaneous abortions. Conception was achieved in two of five patients with DQα compatibility.

To date, no study has been performed to evaluate the efficacy of IVIg treatment in patients with recurrent IVF failure and marked genetic similarity. Since this is a small minority of couples being treated, a positive effect may not be expressed in previous studies encompassing diverse study groups. This work describes the use of this modality for treating this unique group of patients.

Materials and methods

Patients

The study group comprised 10 patients between the ages of 28 and 41 years who were enrolled in the IVF programme at the Hadassah University Hospital in the years 2000–2004. All patients underwent IVF for indications other than recurrent spontaneous abortions and had failed implantation following seven or more cycles of fresh embryo transfers with at least two embryos of good quality. These patients were identified from 162 couples (6%) with recurrent IVF failure who underwent HLA testing, and were found to share at least three HLA loci (two alleles of each A, B, C, DR, DQ) with their partner, and had a negative crossmatch test with him.

HLA class I and II typing

Serological typing was performed using the Complement Dependent Microlymphocytotoxicity National Institute of Health Extended technique (Terazaki et al., 1978) with some modifications.

Genomic DNA was isolated by means of the salting out method (Miller et al., 1998). HLA-A, B, C, DRB1 and DQB1 loci were typed using the Lipa HLA kit (Murex Innogenetics, Ghent, Belgium). In brief, the Lipa HLA tests are based on the reverse hybridization principle (Buyse et al., 1993). Amplified biotinylated DNA material by polymerase chain reaction (PCR) was chemically denatured, and the single strands were hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. After hybridization, streptavidin labelled with alkaline phosphatase was added and bound to any biotinylated hybrid previously formed. Incubation with BCIP/NBT (alkaline phosphatase substrate solution) resulted in a purple/brown precipitate. The reaction was stopped by washing and the reactivity pattern of the probes was recorded.

One woman was found to be a heterozygote carrier of the factor 5 Leiden mutation, and was treated with low molecular weight heparin during earlier attempts following oocyte aspiration as well as during her current pregnancy. No further thrombophilies were identified.

IVIg treatment

Patients received two courses of 30 g IVIg (Omrigam; Omrix Biopharmaceuticals Ltd, Givat Shmuel, Israel). The first dose was given prior to oocyte retrieval, on an outpatient basis. The second dose was given as soon as a fetal pulse was detected by ultrasound.
Results

Table 1 presents data regarding patient’s prior fertility history. All couples had undergone at least seven previous failed IVF trials, with an average of 9.8 trials (range 7–14) with no pregnancies. In total, these couples had undergone a total of 98 IVF cycles prior to enrolling in this study. An average of 4.3 identical HLA loci were found amongst the couples, two couples shared five loci and two six and seven loci each. Table 2 presents results of IVIg treatment. Seven patients conceived (70%), and two patients conceived twice. Following 18 IVIg treated cycles, a total of nine pregnancies were achieved, for a pregnancy rate of 50% per treatment cycle. Of these pregnancies, five ended beyond 27 weeks, resulting in the delivery of seven healthy newborns (one newborn born at 27 weeks, died following complications of prematurity). One twin pregnancy is currently ongoing, with the patient in the early third trimester.

Table 1. Infertility history of 10 patients in the study group.
IVIg = IV immunoglobulin; PCO = polycystic ovaries.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at treatment (years)</th>
<th>IVF indication</th>
<th>No. IVF courses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>Mechanical</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>Mechanical, PCO</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>Unexplained</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>Unexplained</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>Unexplained</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>Unexplained</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>Male infertility</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>Male infertility</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>Male infertility</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>39</td>
<td>Male infertility</td>
<td>14</td>
</tr>
</tbody>
</table>

*Prior to IVIg, with embryo transfer.

Table 2. Results of IVIg treatment.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>HLA compatibility (no. loci)</th>
<th>Pregnancy from IVIg</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1, 2</td>
<td>1 = twins, amnionitis 19 weeks; 2 = twins, 33 weeks</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1</td>
<td>Abruptio 19 weeks</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1</td>
<td>Normal vaginal delivery, 39 weeks</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>1</td>
<td>Caesarean section, 38 weeks</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>1</td>
<td>Twins, Caesarean section, 27 weeks</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>1, 2</td>
<td>1 = spontaneous abortion; 2 = triplets, Caesarean section, 34 weeks</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>1</td>
<td>Normal vaginal delivery, 37 weeks</td>
</tr>
</tbody>
</table>

*0 = none, 1 = first course of treatment, 2 = second course of treatment.
Discussion

Recurrent IVF failure is one of the more enigmatic and frustrating conditions in fertility treatment. Treatment options are limited, with few alternatives having a significant effect on results. Clinical observations suggest that immune disparity could be a factor in fecundity and in success of assisted reproductive treatment (Daunter, 1992; Weetman, 1999; Szekeres-Bartho, 2002). This aspect of infertility is a controversial one and is still being investigated.

The last decade has found the use of IVIg spreading to the field of IVF. Though several studies seem to demonstrate that IVIg may not be a relevant routine treatment for failed IVF (Coulam et al., 1994; De Placido et al., 1994; Stephenson and Fluker, 2000), there is only scarce data regarding the use of this mode of treatment specifically for HLA compatible couples (Balasch et al., 1996; Scher et al., 2000). The data seem to show an improvement in pregnancy rates among these patients. High pregnancy rates were specifically achieved among couples undergoing IVF for indications other than recurrent spontaneous abortions. It is possible that the immune pathogenesis of RSA is not solved by IVIg treatment, as presented in the literature, nor when undergoing IVF for this indication. Couples who will not benefit from IVIg treatment for RSA may not benefit from this mode of treatment when undergoing IVF either. Nevertheless, couples suffering from recurrent IVF failure, with no prior history of spontaneous abortions, and evidence of HLA similarity, are more likely to benefit from IVIg treatment.

Severe side effects of IVIg treatment are rare. Patients may suffer from malaise, fever and headache (Beer and Kwak, 2001). In the present study, one woman suffered from dizziness.

IVIg is a relatively expensive mode of treatment; the cost for one course of treatment is $2000 US (for both doses), preventing wider use of this preparation for experimental indications outside study groups.

The data include to date only ten couples who met the inclusion criteria regarding number of prior IVF failures and sufficient HLA similarity. Another difficulty is the lack of a control group besides the comparison between past IVF trials and the IVIg treated courses. Though these promising preliminary results suggest that HLA typing should be considered for all couples with recurrent IVF failure, a future prospective randomized multi-centre trial on a larger scale is necessary to confirm this statement. Recurrent IVF failure is a clinical definition that includes several subgroups, each having a unique underlying mechanism. It is suggested that one such subgroup includes patients with HLA matching that may interfere with implantation. Proper identification of these couples may help select those who might benefit from IVIg treatment.


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