Does Immunotherapy for Treatment of Reproductive Failure Enhance Live Births?

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Keywords
Immunotherapy, intralipids, intravenous immunoglobulin, reproductive failure

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Submission January 20, 2012;
accepted January 23, 2012.

Citation
Coulam CB, Acacio B. Does immunotherapy for treatment of reproductive failure enhance live births? Am J Reprod Immunol 2012; 67: 296–303

doi:10.1111/j.1600-0897.2012.01111.x

Before effective treatment for reproductive failure can be instituted, the cause of the failure must be determined. A search of PubMed was made to identify the published data regarding diagnosis and treatment of reproductive failure. Results were compared with the frequency of antiphospholipid antibodies (APA) in 2995 women with histories of unexplained infertility, recurrent implantation failure, recurrent pregnancy loss, and fertile women. In addition, pregnancy outcomes among 442 women experiencing reproductive failure and elevated NK cell activity after treatment with intravenous immunoglobulin (IVIg) ($N = 242$) or intralipids ($N = 200$) were compared. The prevalence of APA was the same among women with the diagnosis of unexplained infertility, recurrent implantation failure, and recurrent miscarriage. Heparin and aspirin are successful in the treatment of elevated APA among women with recurrent miscarriage but not with recurrent implantation failure. IVIg has been successful in the treatment of recurrent miscarriage and recurrent implantation failure among women with elevated APA and/or NK cell activity. When the pregnancy outcomes of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid were compared with women treated with IVIg, no differences were seen. Immunotherapy for treatment of reproductive failure enhances live birth but only in those women displaying abnormal immunologic risk factors.

Introduction

Failure to reproduce is a physically and emotionally challenging ordeal. When reproductive failure is repetitive, these feelings are magnified. Effective treatment for reproductive failure depends on the cause. Thus, the answer to the question 'Does immunotherapy for treatment of reproductive failure enhance live births?' is no, UNLESS there is an immunologic cause of the reproductive failure. Before effective treatment can be instituted, the cause of reproductive failure must be determined. The most frequently studied risk factors to identify an immunologic cause of reproductive failure have included the presence of antiphospholipid antibodies (APA)[1,2] and elevated natural killer (NK) cell killing.[1,3]

Reproductive failure is surprisingly common with up to 75% of fertilized eggs not producing live births.[4] These 75% of pregnancy losses can be classified according to the time during pregnancy when the loss occurs: preimplantation, peri-implantation and post-implantation. Clinically, individuals experiencing recurrent preimplantation pregnancy loss present as unexplained infertility, peri-implantation failure as recurrent implantation failure after *in vitro* fertilization (IVF) and embryo transfer (ET) including chemical (also called preclinical and occult) pregnancy loss, and post-implantation failure as recurrent miscarriages. Infertility has been defined as the failure to achieve pregnancy after 12 months or more of regular unprotected intercourse, and unexplained infertility is a diagnosis of exclusion when

the standard investigation of both male and female partner has ruled out other infertility diagnoses.[5] Recurrent implantation failure has been defined as a cumulative of eight cleaved embryos transferred or four blastocysts transferred with human chorionic gonadotropin (hCG) serum concentrations <5 mIU/mL 14 days after ET.[6] Recurrent pregnancy loss has been defined by the American Society for Reproductive Medicine as two or more failed pregnancies.[5]

While the role of APA in the pathophysiology of recurrent pregnancy loss is well established, the importance of APA in infertility has been controversial, especially among women undergoing IVF/ET. We, therefore, will review the prevalence and treatment of APA among women with a history of unexplained infertility and recurrent implantation failure after IVF/ET as well as patients experiencing recurrent pregnancy loss.

Elevated levels of NK cell cytotoxicity have been linked to increased rates of spontaneous abortions and to IVF failure.[7–9] Increased killing activity can be the result of elevated numbers of NK cells or increased cytotoxicity within each cell. Detection of elevation of circulating CD56+ (NK) cells and NK cell activity has been shown to be helpful in identifying individuals at risk for not implanting embryos[9] and for losing karyotypically normal pregnancies.[10] Treatment outcomes for these patients will also be reviewed.

Prevalence of antiphospholipid antibodies among women experiencing unexplained infertility, recurrent implantation failure, and recurrent pregnancy loss

The prevalence of APA among women experiencing reproductive failure has been accepted to be between 20% and 30%.[1,11] Fig. 1 compares the frequency of APAs among 1325 women with a history of unexplained infertility, 676 with recurrent implantation failure, 789 experiencing recurrent pregnancy loss, and 205 fertile control women. When compared to fertile control women, significant differences in the prevalences of antiphospholipid antibodies in the IgG (Fig. 1a) and IgM (Fig. 1b) but not IgA (Fig. 1c) isotypes were noted. All of the IgG

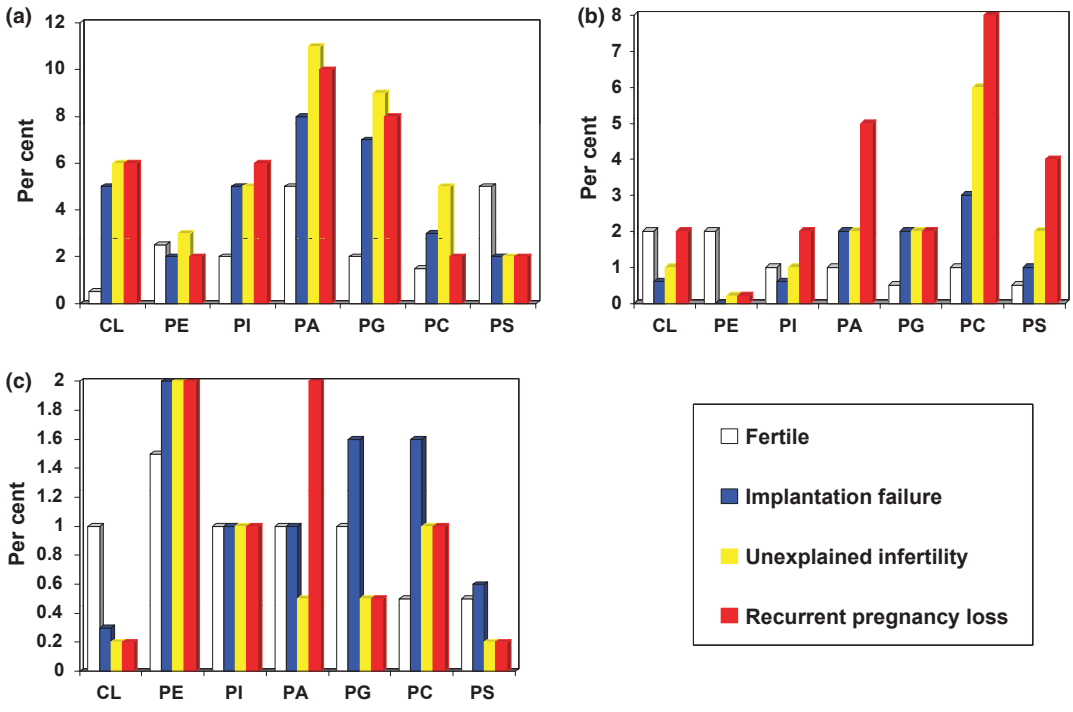

Fig. 1 Percentage of abnormally elevated antiphospholipid antibody serum concentrations including anticardiolipin antibodies (CL), antiphospatidylethanolamine (PE), antiphosphatidylinositol, antiphosphatidic acid (PA), antiphosphatidylglycerol (PG), antphopatidylcholine (PC), and antiphosphatidylserine (PS) among fertile women (white bar), women experiencing recurrent implantation failure (blue bar), women with unexplained infertility (yellow bar), and women with a history of recurrent pregnancy loss (red bar). a = IgG; b = IgM; and c = IgA antiphospholipids.

antiphospholipid antibodies studied were significantly elevated over fertile control values except for antiphosphatidyl ethanolamine and antiphosphatidyl choline (Fig. 1a). The frequency of antiphosphatidyl choline was significantly increased among women with unexplained infertility, recurrent implantation failure, and recurrent pregnancy loss in the IgM isotype when compared with fertile control women (Fig. 1b). No significant increases in prevalence were noted in the other IgM antiphospholipid antibodies. No differences were observed with the prevalence of IgA antiphospholipid antibodies when women with a history of unexplained infertility and recurrent implantation failure were compared with women experiencing recurrent pregnancy loss (Fig. 1c).

The frequency of women displaying one or more than one positive antiphospholipid antibody is shown in Fig. 2. Between 8% and 9% of women with a history of unexplained infertility and recurrent implantation failure had more than one positive antiphospholipid antibody compared with 1.5% of fertile negative control women ($P = 0.0001$) and 11% of women experiencing recurrent pregnancy loss (positive control) (P = NS). Among the 260 women with a history of reproductive failure who displayed more than one positive antibody, 138 or 53% were negative for anticardiolipin antibodies.

Even though all investigators who have studied antiphospholipid antibodies among women experiencing reproductive failure have shown an increased prevalence compared with those with no such

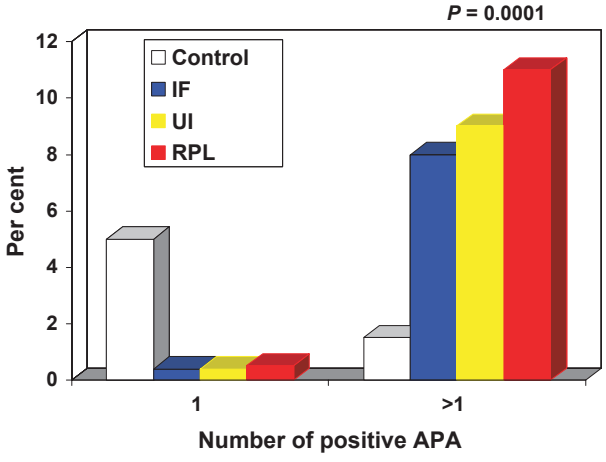

Fig. 2 Frequency of one or more than one positive antiphospholipid antibody among fertile women (white bar), women experiencing recurrent implantation failure (blue bar), women with unexplained infertility (yellow bar), and women with a history of recurrent pregnancy loss (red bar).

finding,[12–21] the relevance of the presence of antiphospholipid antibodies on subsequent pregnancy outcome has been questioned, especially among women undergoing *in vitro* fertilization (IVF) and ET for treatment of infertility. Four studies have shown an adverse effect of the presence of antiphospholipid antibodies on pregnancy rates after IVF,[12–15] five studies[16–20] have shown no correlation, and one study[21] showed a beneficial effect between the presence of antiphospholipid antibodies and implantation or pregnancy rates following IVF. *In vivo* studies in mice have shown an effect of APA on both the preimplantation embryo and the uterus resulting in decreased implantation.[22] The degree to which APA decreased implantation rates by affecting the preimplantation embryo and uterus was determined by doing crossover ET experiments. The major effect contributing to decreased implantation rates appears to be on the preimplantation embryo.[22] Preimplantation embryos exposed to antiphospholipid antibodies *in vivo* did not implant even if they were transferred into a uterus not exposed to antiphospholipid antibodies. A detrimental effect of antiphospholipid antibodies upon preimplantation human embryos has also been shown. Women with elevated antiphospholipid antibodies undergoing IVF/ET have a higher frequency of morphologically abnormal preimplantation embryos than women without elevated antiphospholipid antibodies.[23] Thus, antiphospholipid antibodies have been shown to have three targets: endothelial cells,[24] trophoblastic cells,[25–29] and preimplantation embryos.[30] The clinical manifestations of antiphospholipid antibodies depend on the impact on its target. The effect of antiphospholipid antibodies on endothelial cells would result in blood clotting and decreased angiogenesis resulting in a clinical presentation of recurrent pregnancy loss or recurrent implantation failure. Inhibition of trophoblastic cell differentiation, proliferation, or invasion presents clinically as implantation failure. A direct toxic effect on the preimplantation embryo would result in unexplained infertility or implantation failure after IVF and ET.

Treatment for women with antiphospholipid antibodies experiencing reproductive failure

Various forms of immunotherapy have been introduced to treat couples experiencing recurrent pregnancy loss. Understanding the mechanisms involved in recurrent pregnancy loss allows a more focused

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approach to specific treatment. While the mechanism by which antiphospholipid antibodies impact the frequency of recurrent miscarriage is thought to be related to clotting of placental vessels,[24] the methods involved in recurrent implantation failure appear to involve the effects on trophoblast differentiation,[25–27] proliferation,[28,29] and migration.[26,27] All of these effects can be mediated through activation of complement.[31] Antiphospholipid antibodies have also been shown to have a direct embryotoxic effect on preimplantation embryos[30] allowing an explanation for the clinical manifestation of unexplained infertility and implantation failure after *in vitro* fertilization and ET.

Heparin has also been used in conjunction with aspirin to prevent placental clotting. The rational for using heparin is that it is a blood thinner and inhibits clot formation by a different pathway than the aspirin. The combination of both heparin and aspirin given to women experiencing recurrent pregnancy loss who had antiphospholipid antibodies is associated with a live birth rate of 80% compared with a live birth rate of 44% in women receiving aspirin alone.[32,33] Live birth rates with heparin, aspirin, and prednisone are 74%.[34] Thus, no enhancement of live birth rates is noticed when prednisone is added to heparin and aspirin therapy for treatment of recurrent pregnancy loss.

As angiogenesis is necessary for uterine receptivity and implantation[35] and as antiphospholipid antibodies inhibit angiogenesis,[36] the effect on the uterus could be mediated through the process of angiogenesis. However, recent studies have shown no effect of heparin on angiogenesis,[37] and past studies have shown no effect of heparin on trophoblast.[25,26,29] Indeed, the effects of heparin on pregnancy rates after IVF/ET are shown in Table I. The only study that used heparin in both APA-positive and APA-negative patients indicated no effect of heparin. Thus, while heparin and aspirin appear to be effective treatment for patients experiencing recurrent miscarriage, they do not appear to have an effect on patients with implantation failure. Another approach to treatment of elevated APA in women with recurrent implantation failure is needed.

Table I Pregnancy Rates (%) Among Women Undergoing IVF/ET Who were antiphospholipid antibodies (APA) Positive and APA Negative and Who were and were not Treated with Heparin and Aspirin (H/A)58

	APA positive		APA negative		
Study	H/A+	H/A−	H/A+	H/A−	P
Kutteh[18]	53	47	75	50	NS
Schenk[59]	51			50	NS
Sher[15]	46	17		27	<0.05
Kowalik[20]		59		58	NS
Birdsall[16]	40			28	NS
Gleicher[17]	50			48	NS
Birkenfeld[12]	9			27	0.02
Stern[21a]	9[a]	9[a]	5[a]	5[a]	<0.0002

IVF, *in vitro* fertilization; ET, embryo transfer.
[a]Implantation rates.

Pregnancy outcome among women experiencing reproductive failure after treatment with IVIg

A number of studies have reported pregnancy outcomes after treatment for recurrent spontaneous abortions and recurrent implantation failure after *in vitro* fertilization (IVF) and ET with intravenous immunoglobulin (IVIg), and results have been summarized in a review by Clark et al.[38] Of the ten controlled trials of IVIg for treatment of recurrent pregnancy loss, four report significant enhancement in the live birth rate with IVIg treatment and six were unable to show benefit of treatment. Five trials gave IVIg before conception and four of the five showed significant benefit in enhancing live birth rates, whereas five trials delayed treatment until pregnancy was established and of those none demonstrated benefit of treatment ($P = 0.04$, Fisher's Exact test).[38] Among the trials showing benefit of treatment with IVIg, three of four used immunologic test results including elevated APA to select patients for IVIG treatment, and among trials showing no benefit from treatment, 0/6 selected patients for treatment using immunologic testing ($P = 0.03$).[38] By waiting until 5–8 weeks of pregnancy to begin treatment, women with pathology occurring earlier would have been excluded and those pregnancies destined to succeed would be included, leading to for selection bias. A negative correlation with delay in treatment is significant. Only one study took into account the pregnancies loss as a result of chromosomal abnormalities. Approximately 60% of the pregnancies lost in the clinical trial would be expected to have chromosomal abnormalities that would not be corrected by IVIg.[39] It has also been recently shown IVIG can increase the success rate in IVF failure patients based on meta-analysis of con-

trolled clinical trials provided treatment is given prior to conception/ET, and a value for immunologic testing is suggested by higher rates of efficacy in patients selected for treatment using these tests.[38] When the recurrent miscarriage and recurrent implantation failure trials were combined, seven of nine pre-conception IVIG trials were successful, and 0/5 post-conception ($P = 0.042$). Among the positive trials, 4/7 used immune tests to select patients for IVIG treatment, and among negative trials, 0/7 selected patients for treatment using immune testing ($P = 0.035$).[28] In addition to elevated APA, elevated circulating levels of NK (CD 56+) cells and increased NK cytotoxicity have been shown to predict successful pregnancy after treatment with IVIg.

Elevated levels of circulating NK cells have been linked to reproductive failure.[1,39–42] Women with a history of multiple prior implantation failures after IVF/ET characterized by a negative pregnancy test[1] and chemical pregnancies[40] after ET demonstrated significantly higher circulating levels of CD56+ NK cells than normal fertile controls. Moreover, when circulating levels of CD 56+, NK cells were measured on the day of ET; the percentage of the CD56+ was significantly higher among women who failed to implant that cycle compared with those who implanted.[9] In addition, elevated percentages of circulating NK cells during pregnancy have been shown to predict loss of karyotypically normal pregnancies and a normal level associated with loss of embryos that are karyotypically abnormal.[39] Aoki et al.[41] showed that high peripheral blood NK activity was associated with an increased risk of miscarriage when the woman subsequently married and tried to reproduce, and this result has been reproduced by Yamada et al.[42] Importantly, 50% of the women had increased NK cell levels, which is strikingly similar to the proportion of recurrent miscarriages that are of normal embryonic karyotype.[39] Taken together, these data suggest increased numbers and/or activity of circulating blood-type NK-related cells can translate into an unfavorable environment at the feto-maternal interface.

Suppression of NK cell activity with IVIg has been previously reported both *in vitro*[43,44] and *in vivo*.[45] The mechanisms by which IVIg are believed to enhance live birth rates include[46]:

- IVIg decreases killing activity of NK cells
- IVIg increases the activity of suppressor T cells
- IVIg suppresses B cell production of autoantibody
- IVIg contains antibodies to antibodies or anti-idiotypic antibodies
- IVIg actions on Fc receptors including binding of complement by the Fc component of IgG

Recently published meta-analyses indicate that IVIG significantly increased the probability of taking home one or more babies patients undergoing IVF for infertility and/or early pregnancy loss in individuals displaying immunologic risk factors including elevated APA and NK cell activity.[38]

Pregnancy outcome among women experiencing reproductive failure after treatment with intralipid

A number of studies suggest that intralipid may modulate immune function including suppression of NK cytotoxicity[47,48] and pro-inflammatory cytokine generation.[49,50] Intralipid has more recently been shown to suppress NK cytotoxicity with equal efficacy as IVIg in an *in vitro* assay[44] as well as *in vivo*.[51]

Pregnancy outcomes of 200 women experiencing recurrent reproductive failure (162 with a history of recurrent implantation failure and 38 with recurrent pregnancy loss) who had elevated NK cell activity and who were treated with intralipids are shown in Fig. 3. No differences in pregnancy rate per cycle of treatment, abortion rate, or livebirth/ongoing pregnancy rates between women with a history of

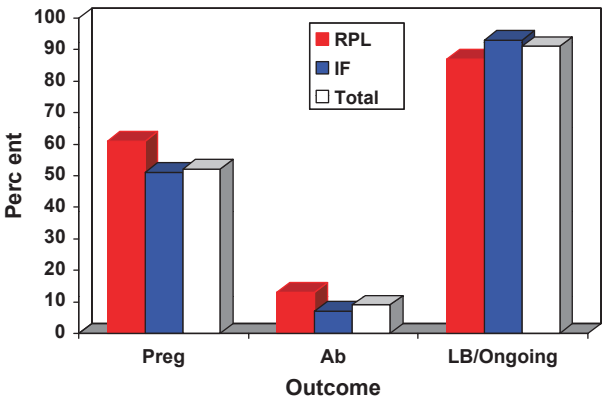

Fig. 3 Pregnancy rates, abortion rates, and livebirth/ongoing pregnancy rates beyond the first trimester among women experiencing recurrent pregnancy loss and recurrent implantation failure who had elevated NK cell activity and who were treated with intralipid. Recurrent pregnancy loss (light blue bars), recurrent implantation failure after IVF/ET (dark blue bars), and total reproductive failure (green bars). IVF, *in vitro* fertilization; ET, embryo transfer.

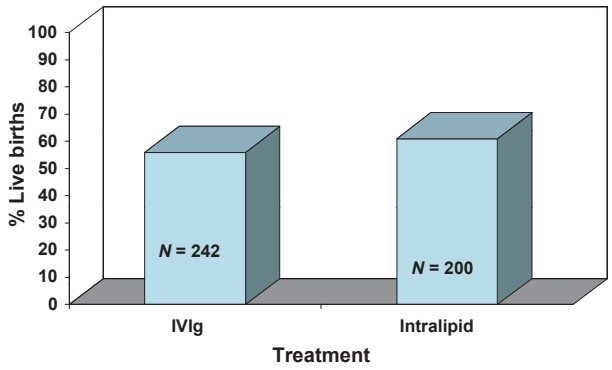

Fig. 4 Comparison of live birth rates of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid and IVIg.

recurrent pregnancy loss and recurrent implantation failure after IVF/ET were noted. The pregnancy rate per cycle of treatment with intralipid for women experiencing reproductive failure with elevated NK cell activity was 52%. Of those who became pregnancy, the abortion rate was 9% and livebirth/ongoing pregnancy rate was 91%.

While the mechanism by which intralipids suppresses NK function is not known, effects of fatty acids have been demonstrated to be mediated through receptors such as peroxisome proliferator-activated receptors (PPARs),[52] G-protein-coupled receptors,[53] and CD1 receptors.[54] Furthermore, intralipids have been shown to stimulate the reticulo-endothelial system and remove 'danger signals' that can lead to pregnancy loss.[55] Sedman et al.[56] have found a significant fall of NK activity and lymphokine-activated killer activity after total parenteral nutrition regimens with long-chain triglycerides. Parenteral fat emulsions are known to accumulate in macrophages and to impair various functions of macrophages and those of the reticuloendothelial system. It was shown that the administration of fat emulsion, Intralipid 20%, to recipient mice can suppress NK cell activity probably through the impairment of the macrophage function.[57]

When the pregnancy outcomes of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid were compared with age- and indication-matched women treated with IVIg, no significant differences were seen (Fig. 4). The overall livebirth/ongoing pregnancy rate per cycle of treatment was 61% for women treated with intralipid and 56% with IVIg.

Summary

Elevated APAs are equally prevalent among women experiencing unexplained infertility, recurrent implantation failure, and recurrent pregnancy loss. Heparin and aspirin are successful in the treatment of elevated APA among women with recurrent miscarriage but not with recurrent implantation failure. IVIg has been successful in the treatment of recurrent miscarriage and recurrent implantation failure among women with elevated APA and/or NK cell activity. Intralipid is effective in the treatment of women experiencing reproductive failure who display elevated NK cell activity. The question at hand is 'Does immunotherapy for treatment of reproductive failure enhance livebirths?' The answer to the question is yes, BUT a treatment is more likely to work if it is given to those with physiological abnormality that the treatment can correct, and, if the treatment in fact corrects it.[58] IVIg and intralipid are both effective in suppressing NK cell cytotoxicity and enhancing live births among women experiencing reproductive failure who display elevated NK cell activity. The results suggest that intralipid can be used successfully as a therapeutic option to modulate abnormal NK activity in women with reproductive problems.

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