

Immunologic Implantation Failure: A Rational Basis for Treatment.

The implantation process begins six or seven days after fertilization of the egg. At this time, specialized embryonic cells (i.e., trophoblast), which later becomes the placenta, begin growing into the uterine lining. When the trophoblast and the uterine lining meet, they, along with immune cells in the lining, become involved in a "cross talk" through mutual exchange of hormone-like substances called cytokines. Because of this complex immunologic interplay, the uterus is able to foster the embryo's successful growth. Thus, from the earliest stage, the trophoblast establishes the very foundation for the nutritional, hormonal and respiratory interchange between mother and baby. In this manner, the interactive process of implantation is not only central to survival in early pregnancy but also to the quality of life after birth.

Considering its importance, it is not surprising that failure of proper function of this immunologic interaction during implantation has been implicated as a cause of recurrent

miscarriage, late pregnancy fetal loss, IVF failure, and infertility. A partial list of immunologic factors that may be involved in these situations includes anti-phospholipid antibodies (APA), antithyroid antibodies (ATA), and, perhaps most importantly, activated natural killer cells (NKa). Presently, these immunologic markers can be adequately measured by only a few (less than a half dozen) highly specialized reproductive immunology laboratories in the United States, from patient blood samples.

1) Natural Killer (NK) Cells

After ovulation and during early pregnancy, NK cells comprise more than 70% of the white blood cell population seen in the uterine lining. NK cells produce a variety of local hormones known as TH-1 cytokines. Uncontrolled, excessive release of TH-1 cytokines is highly toxic to the trophoblast and endometrial cells, leading to their programmed death (apoptosis) and, subsequently to failed implantation. In the following situations these NK cells can become abnormally activated, and thereby produce these TH-1 cytokines:

v When both male and female share specific DNA (DQ-alpha) similarities. In such cases, the presenting problem is usually recurrent pregnancy loss, rather than "infertility".

v In female patients that have both pelvic disease and abnormal APA testing

v In about half of the cases where the woman forms antibodies against her own thyroid gland (i.e., antithyroid antibodies.)

Activated NK cells (NKa) can spill over from the uterine lining into the peripheral blood where their toxicity can be measured. Intralipid therapy, initiated more than 1week prior

to embryo transfer, can subdue activated NK cells, thereby reducing the risk of implantation failure.

2) Cytotoxic Lymphocytes (CTLs).

CTLs release "toxins"(perforins and granzymes) and TH-1 cytokines that counter the humoral, TH-2 cytokine response that is a necessary prerequisite for B-cells to produce

antibodies. The “toxins” and TH-1 cytokines damage or kill the cells that form the outer layers of the embryo’s root system”(i.e.; the trophoblast). By pitting their TH-1 response against the counter-effect of humoral TH-2 cytokines, both CTL’s and activated NK-cells (Nka) regulate and control the degree to which the trophoblast (placenta) invades the uterine wall as well as the tolerance and acceptance by the uterus of the foreign fetal “transplant” (allograft). Studies have shown that women who experience recurrent pregnancy miscarriages have significantly raised levels of CTL’s, Nka’s and TH-1 Cytokines (the so called “embryotoxic factor”) in their uterine linings as well as in the peripheral blood.

4) Antithyroid Antibodies (ATA)

A clear relationship has been established between ATA and reproductive failure (especially recurrent miscarriage and infertility).. About 50% of women who harbor ATAs also test Nka positive. The risk of implantation failure in ATA positive women appears to be confined to cases where ATAs coexist with Nka+.

5) Alloimmune Implantation Dysfunction: HLA compatibility; HLA-G and DQ-alpha.

A pregnancy must be recognized as foreign to trigger the appropriate immunologic mechanisms. HLA compatibility plays an important role in this recognition (HLA-A, B, C, DR, DQ and DP). Overall, if the father’s HLA complex too closely resembles the mother’s HLA complex, then this recognition does not occur thus resulting in a spontaneous abortion or implantation failure. HLA-G is a unique HLA that is expressed by cytotrophoblast cells which compose the inner layer of the placenta. The HLA-G isotypes is vital to the maternal tolerance of the fetus and functions as immunosuppressive. In other words, HLA-G serves as a defense mechanism to protect the placenta (embryo) from the maternal NK cells. We recently reported that it is now possible to determine the accurate pregnancy potential of each embryo before IVF implantation. The presence of high levels of sHLA-G had a positive predictive value greater than 70% in women under the age of 39, and over 50% in women from 39 to 44 years old.

Embryonic paternal antigens regulate trophoblastic HLA-G expression which maintains a balanced TH-1: TH-2 release by T-Cell (CD-4) Lymphocytes In the absence of this paternal antigen-induced HLA-G, normal trophoblastic proliferation and embryo implantation cannot occur. When the sperm provider and the embryo recipient share several HLA antigens (e.g HLA, B, C, DR, DQ or DP), there is a break down in HLA-G related cytokine signaling and an imbalance occurs in the TH-1-TH-2 balance with TH-1 cytokines predominating. This often causes progressive or sudden implantation (trophoblastic) failure, most commonly manifesting as recurrent miscarriages and sometimes as unexplained IVF failure.

THERAPEUTIC IMMUNOMODULATION

a) Corticosteroid Therapy (Prednisone, Prednisolone and Dexamethazone)

Steroid therapy is routine in most IVF programs. Some advocates use daily oral methyl prednisolone .We prescribe oral dexamethazone commencing about ten days prior to initiating ovarian stimulation with gonadotropins, and continuing until the diagnosis of pregnancy, whereupon, in the event of a negative test (Beta HCG or ultrasound), the dosage is tapered over a period of seven to ten days, and then discontinued. Pregnant patients continue treatment through the first trimester. Steroids are believed to act by

inhibiting the cellular immune response

b) Lovenox

There is compelling evidence that the subcutaneous administration of lovenox for female causes of infertility who test positive for APAs, but negative for NK activation), significantly improves IVF birth rates. Lovenox administration is withheld on the day of egg retrieval until immediately following embryo transfer. Lovenox is thought to act by repelling APAs from the surface of the trophoblast (early "root system" of the embryo). Provided that platelet counts are normal, are checked on a regular basis, and Lovenox is withheld on the day of egg retrieval, its administration is virtually risk-free.

c) Intravenous Immunoglobulin (IVIg) or Intralipids therapy

Intravenous Immunoglobulin (IVIg) is a sterile protein preparation derived from human blood. Every effort has been made to ensure that it is free of bacterial and viral contamination. There are basically four ways in which IVIg is believed to offset or counter the anti-implantation effects associated with reproductive immunologic deficiencies. *First*, it is a potent suppressor of activated (toxic) Natural Killer cells (NKa). *Second*, IVIg reduces the activity of CTL's (activated T-cells), which are major producers of TH1 cytokines ("toxins") that can damage the early implanting conceptus. *Third*, IVIg is believed to suppress the ability of B cells to produce damaging autoantibodies such as APAs and antithyroid antibodies (ATA) and, *Fourth*, IVIg contains anti-idiotypic antibodies that directly counter many of the damaging effects of autoantibodies (antibodies that attack the body's own cells), such as antiphospholipid antibodies (APAs), thereby protecting the early "root system" of the embryo/conceptus from damage.

IVIg has had some undeserved bad press. Since it is a blood derivative, the thought of administering it in an era where HIV is rampant, is frightening to most. However, consider the following: IVIg products available in the United States and the United Kingdom are subject to the most stringent controls and scrutiny. According to the manufacturers of IVIg, there has not been a single case of HIV viral transmission in more than two million administrations and there have only been a few isolated cases of Hepatitis C. This is not surprising since IVIg is derived from the very same blood pool used for transfusion purposes, and since millions of units of blood have been administered in the United States over the last 7 years without any reports of HIV transmission. The IVIg available in the U.S is thoroughly tested. We hold, that if administered properly by qualified medical personnel, and the appropriate precautions are

taken, IVIg, currently used in this country, is virtually devoid of viral contamination. Intralipid therapy has replaced the use of IVIg over the last year here at SIRM LI. It is not a blood product and is markedly less expensive than IVIg. It has the same NKa deactivation parameters as IVIg and we have had no difference in our success rates treating immune issues since we have transferred to this treatment modality. By incorporating into the cell walls of the NK cells and by transport into the cytoplasm of these cells, the intralipids are able to carry out the deactivating process as effectively as the IVIg.

We recommend that IVIg or intralipids in cases for specific clinical conditions where in

spite of having transferred numerous “good quality embryos” implantation does not occur and there is associated activation of NK cells when there is a known history or family history of Autoimmune disease in association with the woman testing positive for NK cell activation(Nka+). In cases of Alloimmune implantation failure (see above) Isolated Nk cell activation, without one of these associated clinical situations, is not in and of itself an absolute indication for IVIG therapy since some Nka+ women do conceive and then continue with healthy pregnancies... without IVIG therapy. IVIG treatment should be initiated at least 7 days prior to embryo/blastocyst transfer.

The selective use of immunotherapy has, on numerous occasions, enabled us to achieve successful pregnancy in many patients who had previously suffered repeated IVF failures

(4 or more). Many such patients had previously been advised, not to try again with their own eggs. We are able to report IVF births occurring in numerous cases, where the woman had previously experienced more than ten (10) IVF failures. One such case involved a 42-year-old woman who was successful with us (using her own eggs) following 22 consecutive prior IVF failures. We believe that such results could not have been achieved without access to selective immunomodulation.

d)Corticosteroid Therapy (Prednisone, Prenisilone andDexamethazone)

Steroid therapy is a mainstay of most IVF programs. Some programs use daily oral methyl prednisilone while others prescribe oral Dexamethazone commencing about ten days prior to initiating ovarian Stimulation with gonadotropins, and continuing until the diagnosis of pregnancy, whereupon, in the event of a negative test (Beta HCG or ultrasound), the dosage is tapered over a period of seven to ten days, and then discontinued. Pregnant patients continue treatment through the first trimester. Steroids are believed to act by inhibiting the cellular immune response.

Because immunologic problems may lead to implantation failure, it is important to properly evaluate women with risk factors such as:

- Unexplained or recurrent IVF failures
- Endometriosis,
- Unexplained infertility or
- A family history of autoimmune diseases (e.g. rheumatoid arthritis, lupus erythematosus & hypothyroidism).

Rather than being causally linked to implantation failure, the detection of APAs (in women whose infertility is not male factor-related), probably function as markers that point to a population at risk. We have demonstrated that in cases of female infertility, women who test APA positive but Nka negative who are treated with lovenox , experience significantly improved IVF outcomes, while APA/ATA positive women who also test Nka positive experience similar benefit when IVIG is given prior to egg retrieval. “In the pursuit of optimizing outcome with IVF, the clinician has a profound responsibility to make every effort at enhancing the environment for implantation. By so doing, he/she will not only maximize the chance of pregnancy, will also promote the noble objective of enhancing the very quality of the life produced.”

Dr. Braverman specializes in fertility patients with auto-immune problems. For more information please click the link below:

<http://www.birms.com>