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Immunotherapy with paternal lymphocytes for recurrent spontaneous abortion: Experience with 409 patients

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Objective: To report our experience in the treatment of women suffering from recurrent spontaneous abortion, using immunotherapy with the husband's leukocyte antigens. Treated patients were evaluated, and the endpoints were birth or abortion after the third month of pregnancy. **Methods:** Between 1991 and April 2007, we evaluated 1070 women with a history of recurrent spontaneous pregnancy loss in the first trimester. Those showing lack of antibody production against the partner's leukocyte antigens were eligible for treatment, and the screening test was the crossmatch between the patient's and the husband's lymphocytes. Couples with a negative crossmatch were selected. Before immunotherapy, the partners were screened for infectious diseases (HIV, hepatitis B and C, syphilis, cytomegalovirus, malaria and Chagas' disease). If any abnormality were detected, the donor would be a first degree relative, as long as infectious diseases were excluded. No patient was treated with multiple donor cells. Between 1991 and 2004, we used the husband's lysed lymphocytes, and in the past 4 years, we used lymphocyte concentrate, administered intradermally, monthly, for three consecutive months. As soon as the patient became pregnant, we would start new monthly applications, until the pregnancy reached the end of first trimester. Of the 1070 patients, 409 were eligible for evaluating the endpoints: birth or abortion after the third month of pregnancy. All patients were evaluated for other causes of abortion, including auto-antibodies and thrombophilias, and treated as necessary. **Results:** 216 women were treated with lysed lymphocytes, and 195 of them (90.3%) were still pregnant after the fifth month, while 21 (9.7%) presented abortion. 193 patients received lymphocyte concentrate, and 173 of them (88.9%) were able to maintain their pregnancies, while 20 (11%) had losses. Of the total 409 patients, 368 (90.0%) had successful pregnancies. The methods' results showed no statistical significance ($p=0.7923$). A small number (16) was submitted to *in vitro* fertilization before treatment with lymphocyte concentrate. Two of them had pregnancy losses and 14 had successful pregnancies. Adverse effects were limited to local reactions, and no serious events were reported. **Conclusions:** In our experience, immunotherapy for recurrent pregnancy loss with paternal lymphocytes was effective in 90.0% of treated patients, and no serious adverse reactions occurred.

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S3

Seminal plasma hypersensitivity: Case report

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Seminal plasma hypersensitivity is a rare disorder, with around 80 published cases in the English language literature, but none from Latin America. This article reports a case of plasma seminal anaphylaxis in Brazil and reviews the main studied aspects of this disease. After her first term pregnancy, a 31-year-old asthmatic married woman came to us complaining of wheezing, facial edema, rash and loss of consciousness, always immediately after sexual intercourse. Diagnostic investigation showed positive skin prick testing for her husband's seminal plasma and detectable specific IgE in the sera. The couple was advised to use condoms and emergency subcutaneous epinephrine. The most important known risk factor for seminal fluid allergy is atopy. Signs and symptoms occur immediately, minutes or hours after the coitus, and may be localized or systemic. The hypersensitivity mechanism is IgE-mediated, and the responsible antigen has a probable prostatic origin common to all men. The diagnosis is confirmed with positive skin testing to seminal plasma and demonstrable specific IgE in the sera. Treatment requires avoidance of exposure by means of cessation of coitus or correct use of condoms, and immunotherapy may be performed. It is important to state that seminal fluid hypersensitivity is not

a cause of infertility, as successful pregnancies in treated patients have been documented.

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S4

Crossmatch positivity and pregnancy after immunotherapy with paternal lymphocytes for recurrent spontaneous abortion

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Objective: To compare the crossmatch positivity and successful pregnancies after immunotherapy with parental lymphocytes for recurrent spontaneous abortion. **Methods:** We treated 409 patients with history of recurrent spontaneous pregnancy loss in the first trimester using immunotherapy with parental lymphocytes. Those showing lack of antibody production against the partner's leukocyte antigens were eligible for treatment, and the screening test was the crossmatch between the patient's and the husband's lymphocytes. Couples with a negative crossmatch were selected. After three monthly doses of lymphocyte concentrate, some of them had the crossmatch exam repeated, and we compared its positivity and the chance of successful pregnancy or abortion after the treatment. **Results:** Sixty-two couples had their crossmatch exams evaluated after treatment. Twenty-seven of them became positive, and 35 were still negative. Of those who had a positive exam, 6 had successful pregnancies and 2 presented abortion. Of those with a negative exam, 10 became pregnant and 2 had losses. We found no statistical significance between a positive crossmatch and successful pregnancy ($p=0.4429$ —Fischer). **Conclusions:** Crossmatch positivity after treatment for recurrent spontaneous abortion using immunotherapy with parental lymphocytes was not predictive of successful pregnancy in our study group.

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S5

HO-1 up-regulation increases the number of uNK at the fetal-maternal interface

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Heme oxygenase-1 (HO-1) is an anti-apoptotic and tissue-protective enzyme. We previously suggested a protective function for HO-1 after observing diminished levels of HO-1 in human and murine miscarriage. Accordingly, up-regulation of HO-1 by means of cobalt-protoporphyrin (Co-PP) or gene therapy significantly diminished the abortion rate in mice. The mechanisms by which HO-1 would exert its actions are not fully characterized yet. At the beginning of human and murine pregnancy, an important population of cells of the innate immune system invades the uterus. These so-called uterine natural killer cells (uNK) make up to 75% of the lymphocytes within the uterus. Although the mechanisms by which uNK work are not well understood, it is known now that they are necessary for a proper trophoblast invasion and a successful pregnancy outcome. Due to the importance of both HO-1 and uNK at the fetal-maternal interface, we aimed to investigate whether HO-1 acts by regulating the number of uNK invading the uterine tissue. First, we concentrated in characterizing HO-1 and uNK at different time points of pregnancy. We further analysed whether HO-1 up-regulation by Co-PP or gene therapy induces changes in the number and quality of uNK. The expression of HO-1 was determined by immunohistochemistry and qRT-PCR in uterus, placenta and decidua of normal pregnant (CBA/J $\times$ BALB/c) and abortion-prone mice (CBA/J $\times$ DBA/2J). uNK were quantified at the fetal-maternal interface after visualization with DBA-lectin staining. The mRNA expression of perforin, a product of mature NK, was measured by qRT-PCR in uterus, placenta and decidua of normal pregnant and abortion-prone mice. Additionally, we performed *in vivo* studies by up-regulating HO-1 by injection of Co-PP on gestation day (gd) 4 or by adenoviral gene transfer on gd 5. As