Lymphocyte immunotherapy can improve pregnancy outcome following embryo transfer (ET) in patients failing to conceive after two previous ET

J.H. Check, *M.D., Ph.D.*; **J.R. Liss,** *M.T.*; **M.L. Check,** *B.A.*; **A. Diantonio,** *B.S.*; **M. Duroseau,** *B.S.*

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if lymphocyte immunotherapy (LIT) can improve the outcome after embryo transfer (ET) in women failing to have a live delivery after at least two previous attempts.

Methods: Women failing to deliver a live baby despite at least two previous ET cycles at Cooper Center for IVF irrespective of previous failed ET cycles in other centers were offered the option of lymphocyte immunotherapy prior to their next ET. They were subsequently matched to the very next woman having ET but in whom LIT was never offered or was refused. The matching was based on age, number of previous failed ET cycles, type of ET (fresh or frozen), and serum follicle stimulating hormone (FSH) level.

Results: The clinical and viable pregnancy rate was 70.3% and 51.3% for the LIT group vs 45.9% and 16.2% for the controls ($p < .05$).

Conclusions: Lymphocyte immunotherapy may help improve outcome following ET in women with previous failures. The data should encourage a larger multicenter prospective study.

Key words: Lymphocyte immunotherapy; Embryo transfer; Live delivery.

Introduction

Failure to conceive despite several previous embryo transfer (ET) cycles with normal appearing embryos may be related to bad luck, occult embryo defects, or possibly some uterine environmental defect. One of the endometrial abnormalities could be immune rejection of the fetus.

There are some data suggesting that successful implantation may be facilitated by the induction of immunomodulatory proteins which inhibit natural killer (NK) cell cytolysis and favor a shift of TH1 to TH2 cytokines [1]. One of these immunomodulatory proteins is associated with progesterone (P) secretion and is called the progesterone-induced blocking factor (PIBF) [1].

In vitro fertilization-ET (IVF-ET) is expensive and when there are a few failures the couple frequently inquires as to what the reason is for the failures and if there is anything else that can be done to improve the outcome. When presented with the above theoretical etiologies one option of treatment, if one does not want to change gametes or use a gestational carrier, is to consider immunotherapy. Patients were informed of the theoretical benefit of suppressing NK cell activity to allow successful implantation [1]. They were also advised that there was some data suggesting that lymphocyte immunotherapy (LIT) could increase production of PIBF [2].

The study presented here evaluated the outcome of women proceeding to another ET cycle choosing LIT and comparing the outcome to a matched control group not receiving LIT.

Materials and Methods

Some women who had a minimum of two ETs at Cooper Center for IVF without achieving a successful pregnancy were offered LIT. Previous failures in other IVF centers were not counted. The couples were informed that there were no previous data showing the benefit of LIT for improving IVF-ET outcome. The use of LIT based on theoretical benefit alone was approved by the 12-member ethics committee of the Cooper Institute for Reproductive and Hormonal Disorders. The procedure is considered safe, inexpensive, and if helpful, could reduce the need for extra risky and expensive IVF-ET procedures.

Patients having ET choosing LIT were matched with the very next couple not having LIT performed. They had the same number of ETs at Cooper Center for IVF failing to achieve a live delivery, same type of ET (fresh vs frozen), age within two years, baseline serum follicle stimulating hormone (FSH) $\leq$ 11 mIU/ml or $\geq$ 12 mIU/ml and whether intracytoplasmic sperm injection (ICSI) was performed or not.

The leukocytes were obtained from the male partner. The LIT procedure required 8-10 ml tubes of heparinized blood. The blood was diluted with normal saline and then layered over Isoprep and centrifuged at ~200 RPM for 30 minutes. The band of mononuclear cells that formed a distinct band at the interface between the sample layer and lymphoprep solution was then removed using a sterile Pasteur pipette without disturbing the layers. The mononuclear cells were then washed and resuspended in saline. Tuberculin synringes were then filled with 0.6 to 0.75 ml of white cell suspension and this was given to the patient as several intradermal injections.

Revised manuscript accepted for publication October 2, 2004

Only the first ET (fresh or frozen) was evaluated. If a positive beta-human chorionic gonadotropin (hCG) level was obtained, it was repeated. If the beta-hCG level doubled appropriately two more times LIT was given again and finally one more time at eight weeks' pregnancy if the ultrasound showed viability.

Both chi-square analysis and Fisher's exact test were used to compare clinical and delivered pregnancy rates between the LIT treated group and the matched control group.

Results

There were 37 matched pairs evaluated. There were 22 fresh and 15 frozen ETs. The clinical (ultrasound evidence of pregnancy) was 70.3% (26/37) for those receiving LIT vs 45.9% (17/37) for the matched control group ($p < .05$, chi-square analysis). The delivered pregnancy rate was 51.3% (19/37) for the women having LIT vs 16.2% (6/37) for the control group ($p < .01$, Fisher's exact test).

The average number of previous failed ET cycles for these 74 women was 4.3. The mean age for the LIT group was 35.4 ± 6.2 vs 35.2 ± 6.1 for the controls.

Discussion

There have been several studies published since 1986 concerning the use of LIT to prevent miscarriage in women with a history of recurrent spontaneous abortion with some showing benefit, some showing no benefit, and one study suggesting it causes a less favorable outcome. A recent Cochrane database systematic review concluded after evaluating 19 trials of high quality LIT studies that paternal cell immunization provides no significant beneficial effect over placebo in preventing further miscarriage [3]. Thus, the higher clinical pregnancy rate seen with this study is consistent with the possibility that LIT improves implantation and conception more than maintaining established pregnancies. However, these data also showed a dramatic increase in delivered pregnancies once a clinical pregnancy was established compared to the controls suggesting a definite role in also preventing miscarriage.

If LIT helps to produce progesterone-induced immunomodulatory protein, e.g., PIBF, the mechanism may be to induce more P receptors on gamma/delta T lymphocytes that secrete this 34 kDa protein [2]. However, the other essential factor needed to produce PIBF is P. Progesterone-induced blocking factor has been found to be lower in women who subsequently have a miscarriage [4, 5]. However, in women supplemented by extra P, the PIBF difference in aborters vs non-aborters was no longer significantly different [6]. Both the fresh and frozen ET cycles were give extra P supplementation starting in the luteal phase (200 mg progesterone vaginal suppositories twice daily for fresh ETs and the same vaginal dosage plus 100 mg IM daily P for frozen ET cycles). There has only been one study to date comparing the use of P supplementation vs. P and LIT for women with recurrent spontaneous abortion and the P and LIT combined therapy demonstrated a significantly improved outcome [7]. Thus we suspect that most women can have sufficient PIBF by having sufficient or even extra P available. However, some may have insufficient P receptors induced on gamma/delta T cells by the fetal semi-allograft and may thus need a more potent allogeneic stimulus, e.g., LIT.

Since the theory for using LIT involved the induction of PIBF, which hypothetically could inhibit endometrial NK cell cytolytic activity but not necessarily effect NK cell percentages in the blood stream or in the endometrium, or serum cytokine levels, the ethics committee suggested that measurement of these tests not be performed especially at the patient's expense. Since the PIBF assay is experimental and requires a great deal of labor intensive work in its measurement, the decision was made not to compare early first trimester PIBF expression in LIT-treated women vs controls unless the preliminary data was encouraging. These data were collected during the time period of January, 1997 to December 31, 2000. Based on these encouraging data we had planned to start measuring PIBF. However the use of LIT without investigation and new drug approval was banned by the Food and Drug Administration so this could not be done.

These preliminary data will hopefully encourage the establishment of a large prospective randomly conducted trial, preferably multicenter in nature, for patients with failure to have a successful pregnancy despite several previous ETs. If it cannot be performed in the United States, perhaps it will encourage studies in other countries. To our knowledge this is the only study of LIT use in IVF-ET cycles.

References

[1] Check J.H., Szekres-Bartho J., O'Shaughnessy A.: "Progesterone induced blocking factor seen in pregnancy lymphocytes soon after implantation". *Am. J. Reprod. Immunol.*, 1996, *35*, 277.

[2] Check J.H., Arwitz M., Gross J., Peymer M., Szekeres-Bartho J.: "Lymphocyte immunotherapy (LI) increases serum levels of progesterone induced blocking factor (PIBF)". *Am. J. Reprod. Immunol.*, 1997, *37,* 17.

[3] Scott J.R.: "Immunotherapy for recurrent miscarriage". Cochrane Database Syst Rev 01-Jan-2003 (1), CD000112.

[4] Szekeres-Bartho J., Varga P., Retjsik B.: "ELISA test for detecting a progesterone-induced immunological factor in pregnancy serum". *J. Reprod. Immunol.,* 1989, *16,* 19.

[5] Szekeres-Bartho J., Faust Z., Varga P.: "The expression of a progesterone-induced immunomodulatory protein in pregnancy lymphocytes". *Am. J. Reprod. Immunol.,* 1995, *34,* 342.

[6] Check J.H., Ostrzenski A., Klimek R.: "Expression of an immunomodulatory protein known as progesterone induced blocking factor (PIBF) does not correlate with first trimester spontaneous abortions in progesterone supplemented women". *Am. J. Reprod. Immunol.,* 1997, *37,* 330.

[7] Check J.H., Tarquini P., Gandy P., Lauer C.: "A randomized study comparing the efficacy of reducing the spontaneous abortion rate following lymphocyte immunotherapy and progesterone treatment versus progesterone alone in primary habitual aborters". *Gynecol. Obstet. Invest.*, 1995, *39,* 257.

Address reprint requests to:
J.H. Check, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)